Variation in the Time of Run, Sex Proportions, Size and Egg Content of Adult Pink Salmon (*Oncorhynchus gorbuscha*) at McClinton Creek, Masset Inlet, B.C.

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ABSTRACT

The spawning runs of pink salmon to McClinton creek, Masset inlet, B.C., in 1930, 1932, 1934, and 1936, differed little in time of appearance of the first migrant and disappearance of the last. The period occupied for the main portion of each run to reach the spawning beds depended chiefly upon rainfall and freshet conditions. Males occurred in greater numbers at the beginning of every run but a subsequent increased influx of females eventually brought about equality of the sexes in two seasons. In the third the males predominated slightly, and in the fourth the females. The average length and weight of males are consistently greater than those for females in the same year. Usually a significant increase in length occurred in both sexes from the commencement to the end of the run. In some cases a similar gain in weight was demonstrated but in others it was apparently masked by loss in weight consequent upon fasting during the spawning migration. The number of eggs per female in a given year increases with increase in length and weight.

Coincident with an investigation of the natural propagation of the pink salmon (*O. gorbuscha*) initiated in 1928 by the Biological Board of Canada under the author's supervision, an examination was made of the time of run, sex proportions, size, and egg content of the adult fish and of the variations occurring in these characters from migration to migration. The present paper sets forth the information gained from these studies.

Pink salmon are unique in that in general *large* spawning migrations take place in a given locality every second year only. Furthermore these appear in alternate years in the northern and southern areas of British Columbia—in every even-numbered year in the former and in every odd-numbered year in the latter. The boundary line may be placed approximately in the vicinity of Queen Charlotte sound. In the "off" year pinks occur in limited quantities and usually seek out different spawning tributaries from those frequented by the runs of the "on" year. In a few localities, e.g. the Tlell river on the east coast of Graham island, Queen Charlotte islands, annual runs are found, but in every case the migration to the district in the "off" season is much smaller.

In Masset inlet, Queen Charlotte islands, the base for the present investigation, however, pink salmon spawning migrations are strictly biennial and confined to the even-numbered years. No individuals have been discovered in the alternate seasons. The particular stream on which the study was carried out, McClinton creek, is typical of the smaller rivers flowing into the inlet and supports an excellent natural run.

METHODS

Capture of Adult Salmon

Capture of the adult salmon was made possible by means of a wooden fence similar to the hatchery racks employed throughout the province for fish-cultural purposes with the exception of slight modifications to suit the particular conditions in the locality. The structure is illustrated and described in a previous paper (Pritchard 1936).

Figure 1. Apparatus employed in measuring and weighing adult pink salmon at McClinton creek, Masset inlet, B.C. (A—measuring board; B—weighing apparatus).

Measurement of Fish

Length measurements were taken by laying the salmon on a flat board on which were scribed the inch and centimetre scales. The snout was lightly pressed against a cross-piece fixed at zero on both scales. The length from the tip of the snout to the centre of the fork of the tail was then read to the nearest one-eighth of an inch and that from the tip of the snout to the end of the vertebral column to the nearest half-centimetre. Only the former measurements are used in this paper. The fish was then placed head downwards in a canvas-board container and weighed on a Pelouse Dairy scale in pounds and tenths of pounds.

The whole operation required approximately one minute after which the fish was returned to the water above the fence unharmed.

Following the practice established by this journal such readings as are used have been converted from the English to the metric system, the inches to centimetres and the pounds to kilograms.

Illustrations of the measuring and weighing apparatus are shown in figure 1.

Treatment of Data

The measurements for the random samples of fish from each run have been averaged and the probable errors of the averages have been calculated by using the formula: $\text{P.E.}_M = 0.6745 \dfrac{\sqrt{\dfrac{\Sigma\delta^2}{N}}}{\sqrt{N}}$. In the interpretation of the resulting data the following basis has been used:

$\dfrac{\text{Difference between averages}}{\text{Probable error of difference}}$	Probability P	
2.45 to 2.90	0.1 to 0.05	Suggestive of a real difference
2.91 to 3.80	0.05 to 0.01	Probably significant
>3.80	<0.01	Definitely significant

RESULTS

Time of Run

As the author has already pointed out (Pritchard 1936), the life of the pink salmon may be divided arbitrarily into two phases, viz.—"the fresh-water" and "the marine". The former includes the period from the time when the adult salmon enter the streams in the autumn on their way to spawn until their progeny migrate to sea as fry during the next spring. The latter lasts from the time when these fry enter salt water until they appear as adults approximately one and one-half years later at the mouths of the rivers ready to move up and deposit their sexual products. Rarely are the fish encountered during the "marine" phase until they become evident as mature individuals in large schools seeking spawning areas in late June, July and August each year. Thus any examination of the runs is limited to a very small portion at the end of the "marine" phase while the individuals are moving up the inlets to the rivers, and to the "fresh-water" period.

Since, as mentioned above, the run of adult pink salmon to Masset inlet occurs only in the even-numbered years, the data are assembled only for 1930, 1932, 1934 and 1936. In the following tabulation are shown for the years designated the dates at which the first migrant appeared at the fence, the day on which the last was seen, and the beginning and end of the period throughout which more than fifty fish per day were counted.

Year	First Migrant	Main Run*		Last Migrant
		Commencement	Completion	
1930	July 30	Aug. 30	Sept. 29	Sept. 29
1932	Aug. 4	Aug. 27	Sept. 16	Oct. 2
1934	July 25	Aug. 18	Oct. 10	Oct. 11
1936	July 29	Aug. 8	Oct. 5	Oct. 5

*More than 50 fish per day.

Qualitatively the runs had a very similar history. All began in late July or early August with scattered individuals working upstream at high tide and on the first of the ebb in a rather aimless and random way. Ten days to one month later the numbers increased, with the main heavy migration taking place usually in late August, September and early October and finishing rather suddenly.

In a previous paper (Pritchard 1936) it has been shown that the time taken for the pink salmon to reach the spawning beds is directly affected by meteorological conditions. If the weather is dry, most of the fish will remain in salt water for an extended period, only scattered individuals entering the creek at high tide or on the first of the ebb. If, before rainfall occurs, the fish attain a condition in which the eggs and sperms are fully developed, loose and ready to be discharged, they will attempt to ascend the river no matter how little water there may be. In the McClinton creek area, however, where the rainfall amounts to over one hundred inches annually (two hundred and fifty centimetres), drought conditions are uncommon especially in the autumn when the salmon runs occur. Usually, therefore, upstream migration begins whenever freshets take place regardless of the state of sexual development. The number ascending the creek has been found to be positively and significantly correlated with the amount of rainfall and the height of the water. A small run might thus be drawn out because of lack of freshets while a large run might be handled quickly if the freshets were continuous. The fact that the smallest run of 1932 (15,600 individuals) occupied least time from the appearance of the first to the disappearance of the last migrant, and that the largest run of 1934, (155,196 individuals) lasted for the longest period, may therefore be a matter of coincidence.

The appearance of scattered individuals at the beginning of the runs can be explained in two ways. In the first place the pink salmon are just arriving from the open ocean in a bright and silvery condition with the primary and secondary sexual characters little developed, and have not a very definite urge to ascend the stream. Secondly it is usual for the latter part of July and the early part of August to be relatively dry in these areas so that freshets are infrequent. There is thus comparatively little external stimulus to provoke ascent of the stream. Similarly the sudden ending of all the migrations is explicable because of the fact that in late September and early October heavy rains are the rule with the result that the river remains high and the fish move upstream continuously. With no replenishment of the stock off the mouth of the river from the open ocean, the end must of necessity come suddenly. Furthermore the short concentrated early-finishing run of 1932 can be directly attributed to the heavy rainfall at the period, 14.8 inches (37.5 cm.) occurring

from August 31 to September 15, as compared with 6.4 inches (16.3 cm.) between the same dates in 1934, and 5.0 inches (12.7 cm.) in 1936.

Proportion of Sexes

The total counts of pink salmon in each year segregated as to sex are submitted below:

Year	Number of males	Number of females	Total	Percentage Males	Percentage Females
1930	32,955	33,198	66,153	49.8	50.2
1932	8,003	7,597	15,600	51.3	48.7
1934	77,477	77,719	155,196	49.9	50.1
1936	24,221	28,391	52,312	46.3	53.7

By the use of the formula, $\sigma = \sqrt{npq}$ and table B, Pearl (1930), page 439, the significance of the deviations of the sexes in each year from even proportions has been assessed. In 1932 and 1936 the ratio of males to females is statistically different from fifty: fifty while in 1930 and 1934 the variation is too small to be considered significant.

A tentative explanation for part of the excess of females in the run of 1936 may be offered. In that year, because of the large number of pink salmon schooled at the head of the bay, a request was made to have the boundaries or fishing limits moved in to allow the capture of more fish. This inference of local abundance had a tendency to attract more fishermen among whom were a larger number of gill-netters than usual. The latter fished very heavily up to within one day of the closure of the season. Due to the developing humps on their backs and slightly larger size, males were taken more frequently by this method. One catch inspected at the cannery showed a very great preponderance of the sex. Foerster (1936) has also indicated that in rivers such as the Fraser gill nets may be selective of male sockeye. Such a circumstance, it is felt, provides a possible explanation of the deviation from equal numbers of each sex.

For the sockeye salmon, *Oncorhynchus nerka*, similar findings have been made in regard to the proportion of sexes in the migrations proceeding to the spawning rivers. Clemens (1935) reports cases in which the males and females were equal in numbers, others in which the former predominate significantly, and still others in which the latter make up the greatest portion. At Cultus lake in British Columbia Foerster (1929 and 1936) has discovered that there is usually a much larger abundance of females, yet in one year males were more numerous. He points out, however, that the disparity from equal numbers may be due to factors not operative in other localities and gives as an example a very selective method of fishing.

In figure 2 are plotted the cumulated percentages of males and females for the four migrations, 1930, 1932, 1934 and 1936. As an illustration of the interpretation of this figure, on September 15 in 1930 the number of males (14,471) which had passed the fence constituted 21.9 per cent of the total run (66,153) while the number of females (9,626) made up 14.6 per cent.

It is commonly stated that during the spawning runs of anadromous fishes the males arrive first, are present during the early part of the run in greater numbers than the females, which gradually gain upon them and eventually exceed them with the result that the disposition of sexes on the spawning beds is approximately equal. Qualitative observations have demonstrated for the

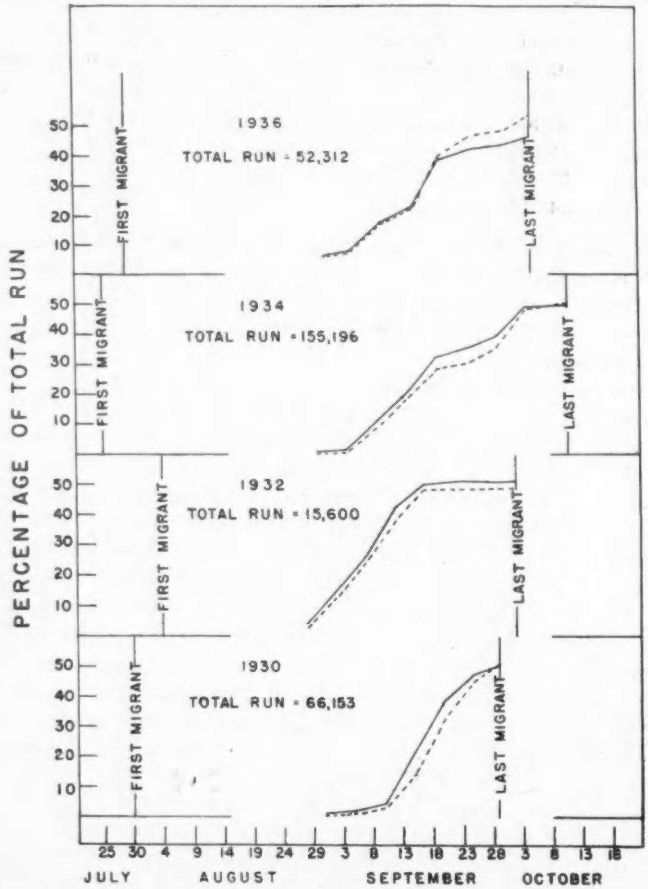

FIGURE 2. Cumulated percentages of males and females for the four migrations, 1930, 1932, 1934, and 1936, at McClinton creek, Masset inlet, B.C. (Solid line—males; broken line—females).

pink salmon at McClinton creek that at the commencement of upstream migration scattered females appear at so nearly the same time as the males that one could not with accuracy assign priority in arrival to either sex. The figure of cumulated percentages does show, however, that the males do arrive in *greatest* numbers in the first portion of every run. At some time later, the

period varying in different years due to the effect of climatic conditions in maintaining the volume of migration, the females arrive in larger quantities and eventually almost equal, or even exceed the males. Thus the pink salmon at McClinton creek approach very closely the generally accepted hypothetical condition set out above.

Size of Individuals

total length

In measuring the fish every effort was made to insure a random sample by dipping fish from either pen without any selection as to sex or size. In addition, measuring was carried out at intervals over the whole period of each migration, the number examined each time being more or less proportional to the numbers appearing, viz.—on days of small runs few were inspected and on days when large runs were in evidence more were measured. The average lengths with their probable errors calculated from the raw data so collected are set down in the table which follows:

	Male		Female	
Year	Number	Average	Number	Average
1930	881	53.7 ± 0.074	543	51.1 ± 0.058
1932	373	57.2 ± 0.112	413	54.3 ± 0.061
1934	417	56.5 ± 0.178	359	52.7 ± 0.076
1936	138	56.2 ± 0.170	170	52.7 ± 0.081

Graphs illustrating the length frequencies in the four years under discussion are shown in figure 3.

Difference between males and females of the same run. From the location of the frequency polygons in figure 3 it appears that the males are on the average consistently larger than the females, the range of length for the former over the four years being 16 to 27½ inches (40.6 to 69.8 centimetres) and for the latter 16½ to 23¼ inches (41.9 to 59.0 centimetres). This assumption is confirmed by the averages in the table above. Adopting the levels of significance given on page 3, it can be shown that in every case the average length of the males is definitely and significantly greater than that for the females of the same year. A similar condition has been reported for sockeye salmon (Foerster 1929, Clemens 1925-1935, and Gilbert 1914-1924).

Variation in length from year to year. When comparison is made between the average lengths for a given sex in different years using the same criterion as above, it is found that the variations are definitely significant except between the figures for 1934 and 1936.

Though it is freely admitted that collection of all statistics concerning the fishery, including the numbers caught, and the total number of adults composing the spawning escapement, would be required for a definite determination of the absolute size of the run to any area, an estimate of the variation from year to year may be obtained from data on the size of the commercial pack and a qualitative estimation of the extent of "seeding", In the case in point it can be definitely and positively stated that 1930 was the year of the largest run

with a pack of approximately 200,000 cases in the inlet, and a heavy escapement to all rivers as shown by the count of 66,153 at McClinton creek. In this year the averages demonstrate that the fish were the smallest. In 1932 the smallest run occurred with a pack of only 2,400 cases and a light spawning as indicated

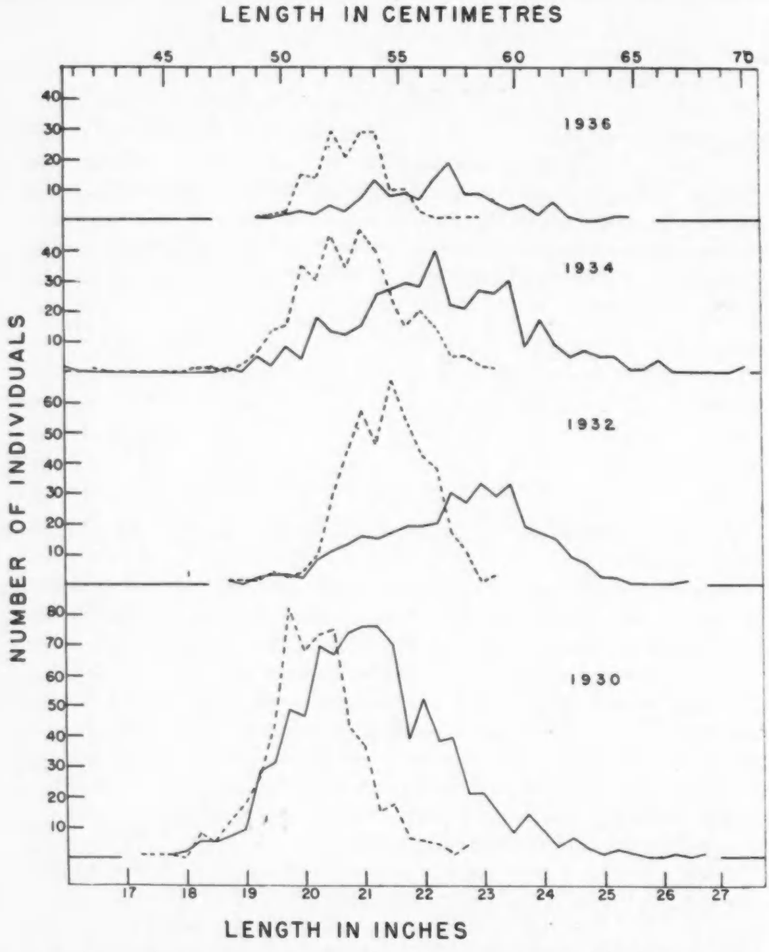

FIGURE 3. Length frequency polygons for adult pink salmon taken at McClinton creek, Masset inlet, in 1930, 1932, 1934 and 1936. (Solid line—males; broken line—females).

from the 15,600 taken in McClinton creek. That season the pink salmon in the McClinton run showed the greatest average length. As far as can be discovered the migrations of 1934 and 1936, while not of exactly the same size, were of the same order of magnitude and were in this respect intermediate between those of 1930 and 1932. The average lengths of the fish were also

intermediate and equal. Therefore either by coincidence or otherwise, it is indicated that in the exceptionally large run the individuals were smaller than those in smaller migrations.

One interpretation of the cause of the variation noted above may lie in a relationship between the density of ocean population and the extent of growth. It does not seem illogical to postulate that each individual in a *large* school of adult pink salmon feeding on the plankton in a localized area would obtain less food than it would if it were in a much smaller school feeding in the same area. Savage (1931) has reported that an analogous condition actually existed for the herring, another plankton feeder, in the Shields area in the North Sea. He states: "In early August the numbers in both plankton and stomach contents had fallen. This can be explained to some extent by the presence in the area of many more herring earlier in the season. Table VIII shows that the landings were at their highest during August, and the demands on the plankton would consequently be heavy. With more mouths to feed, each would get less." Such being the case it might be expected that in a year like 1930 when there was a huge run, the available food for a given stock would be decreased and that this situation would give rise to smaller fish. Conversely in 1932, the year of small population, the fish would be larger. Further research into this phase of the subject may produce very pertinent data concerning the differences in size from year to year.

Variation in length during the migration period. With a view to discovering any variation in size as the run progresses, the average lengths for each *quarter* of the sample for each year together with the probable errors for differences close to significance, or over, have been calculated and are submitted in the table below. In every case the first quarter is first in point of time of run including those fish which came in early. The second, third and fourth quarters are also in chronological order, the last containing those fish measured toward the end of the run.

Year	First Quarter	Second Quarter	Third Quarter	Fourth Quarter
Male				
1930	53.0 ± 0.147	53.3	54.0	54.0 ± 0.122
1932	55.9 ± 0.239	57.2 ± 0.193	57.8	57.8 ± 0.193
1934	55.2 ± 0.208	56.5 ± 0.200	56.8 ± 0.251	58.1 ± 0.251
1936	55.2 ± 0.330	56.2 ± 0.305	56.2 ± 0.330	57.2 ± 0.381
Female				
1930	51.1	51.1	51.1	51.1
1932	54.0 ± 0.116	54.3	54.6	54.6 ± 0.124
1934	52.4 ± 0.114	53.0	53.3	53.0 ± 0.142
1936	52.4 ± 0.142	53.0	53.0	53.0 ± 0.168

For the male pink salmon there is in every year a significant difference in length between the first and fourth quarters of the sample, and in some cases between the first and the second or third quarters. It is concluded therefore,

that *smaller* individuals of that sex arrive in greater numbers at the first of the spawning migration. Such a condition might be in the main attributable to the increase in length which is known to result in Pacific salmon from the development of the long hooked snout as one of the secondary sexual characters. Davidson (1935) who has recently made a special quantitative and qualitative study of this phenomenon in the case of Alaskan pink salmon, states: "The length of the head in the male between a line connecting the posterior extremities of the pterotic bones and a line connecting the anterior margins of the external nares increases approximately 5 per cent due to sexual maturity. The length of the head between a line connecting the anterior margins of the nares and the tip of the snout increases approximately 67 per cent due to sexual maturity".

Such an explanation does not, it is felt, account completely for the increase in size from the beginning to the end of the run at McClinton creek. In this locality where the freshets are usually favourable, the fish move up the river almost as soon as they enter the bay. The result is that the general state of maturity is approximately the same throughout the migration, with silvery fish appearing at all times and few individuals reaching the stage at which milt would be ejected. In this connection Dr. F. A. Davidson of the United States Bureau of Fisheries, Seattle, Washington, has been kind enough to allow the writer to examine and to use some measurements of the body length exclusive of the head (Standard body length, Davidson 1936) for pink salmon from Olive cove, Alaska. In the three years, 1930, 1931, and 1932, there is a slight increase in this measurement with the seasonal progression of the run, which variation, although not of definite statistical significance, is at least suggestive of a real difference.

For females, however, which show very little change in body form due to sexual maturity, the increase in length between the first quarter and either of the second, third or fourth is probably significant in 1932 and 1934 ($P=0.018$ and 0.026 respectively) and suggestive of a real difference in 1936 ($P=0.07$). The lack of increase in 1930 is considered to be bound up with the delayed migration due to paucity of freshets. In the dry weather which occurred early in the season the fish remained for some time off the mouth of the river, presumably mixing thoroughly before the rainfall and resulting freshets almost a month later produced the stimulus to ascend to the spawning grounds.

Concerning the cause of this increase in body length from one quarter of a migration to a later one, it is not illogical to assume that there may be a certain amount of precocious development sexually and otherwise on the feeding grounds in the open ocean which may be responsible for certain fish beginning to migrate inshore earlier. These individuals which will reach the streams first, will naturally be smaller than those which remain feeding for a longer period and assume the resulting growth.

Total Weight

The data have been summarized and assembled and averages for each year are set down in the following table while the frequencies are illustrated in figure 4.

	Male		Female	
Year	Number	Average	Number	Average
1930	172	1.59 ± 0.022	202	1.53 ± 0.012
1932	369	2.32 ± 0.013	348	2.04 ± 0.008
1934	417	1.98 ± 0.012	357	1.70 ± 0.008
1936	136	1.87 ± 0.019	169	1.70 ± 0.010

Difference between males and females of the same run. As in the case of total lengths the frequency polygons illustrate that the males are definitely larger

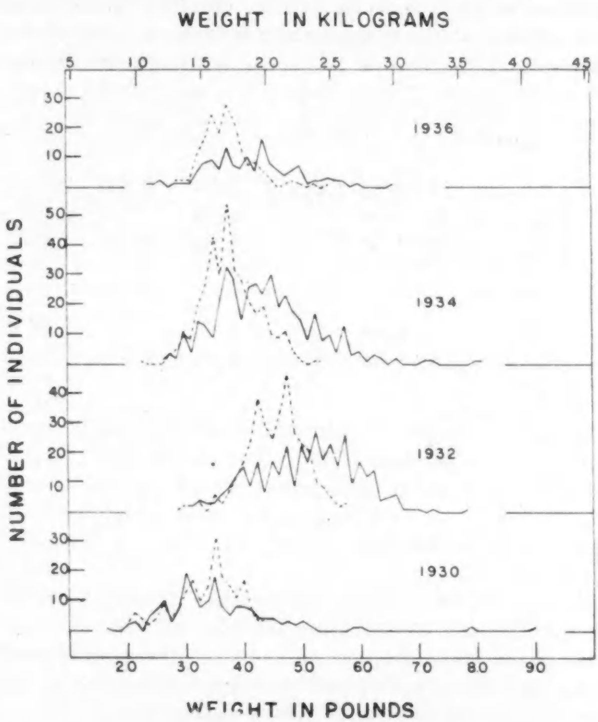

FIGURE 4. Weight frequency polygons for adult pink salmon at McClinton creek, Masset inlet, in 1930, 1932, 1934 and 1936. (Solid line—males; broken line—females).

than the females. The former, over the four years, range from 1 5/8 lb. to 9 lb. (0.74 to 4.08 kg.) and the latter from 2 lb. to 5¾ lb. (0.91 to 2.61 kg.). The averages for each year shown above demonstrate differences between the sexes ranging from 0.125 to 0.625 lb. (0.06 to 0.28 kg.). Of these variations those for 1932, 1934 and 1936 are significant. That for 1930 (0.125 lb. or 0.06 kg.) is merely suggestive of a difference although it varies in the same direction as those for the other years ($P = 0.09$).

Variation in weight of the same sex from year to year. The average weights

for a given sex in separate years differ significantly with the exception of the females for 1934 and 1936. The difference for the males in the two years specifically mentioned above has assumed significance although in the case of the lengths it was too small to be considered. Such variations are probably due, as suggested in the case of the lengths, to changes in the population density on the feeding grounds.

Variation in weight during the migration period. In the table which follows are submitted the average weights for each quarter (used in the same sense as in the case of lengths) of the runs together with the probable errors for those cases where the differences are close to significance or over. The determinations for 1930 are omitted since all the weighing for that year was carried out on a small portion at the end of the run and not randomly distributed throughout.

Year	First Quarter	Second Quarter	Third Quarter	Fourth Quarter
Male				
1932	2.15 ± 0.028	2.32 ± 0.023	2.38 ± 0.027	2.38 ± 0.026
1934	1.93 ± 0.020	1.98	1.98	2.04 ± 0.028
1936	1.81 ± 0.037	1.87	1.93 ± 0.039	1.93
Female				
1932	2.04	2.04	2.04	2.04
1934	1.70 ± 0.014	1.76 ± 0.016	1.76	1.70
1936	1.70	1.70	1.70	1.70

The average weights for the quarters of the samples do not consistently show a significant increase during the course of the run. For the males the difference in 1932 is definitely significant ($P = <0.01$), that for 1934, probably significant ($P = 0.028$). In 1936 the variation is in the same direction but it is merely suggestive of a difference ($P = 0.12$). For the females there is no significant increase.

Such a discovery for the weights is not opposed to the findings in the case of the lengths when all the factors are considered in some detail. Weights of Pacific salmon taken while the fish are on their spawning migration may be somewhat unreliable since feeding has stopped and changes may be taking place in connection with the development of the sexual products about which little is known at present. This must be borne in mind in a comparison such as that given above although it will have little effect in the comparison of fish from year to year or of two sexes in the same year. The figures given in this paper indicate that loss in weight may occur in the pink salmon as they approach the spawning areas. Taking as an example the year 1932, it will be noticed that the length of the females increased from 54.0 to 54.6 centimetres, yet the weight remained the same. Such an occurrence may be due to two causes. In the first place the fish may be losing weight as the run progresses; or, secondly, a new run of long, thinner fish may have come in. All observations lead us to believe that the former is the correct explanation. The conclusion is justifiable, therefore, that there is a definite increase of weight as the run progresses as indicated in at least

two cases, but in some instances this may be masked by a coincident loss in weight due to factors operative during the migration. The cause of this increase is attributed as in the case of lengths to a longer period of feeding in the sea before migration.

Relation of Size to Number of Eggs

In connection with the investigation of the efficiency of natural propagation being carried on at McClinton creek, it was necessary each year to count the number of eggs in a random sample of females in order to arrive at the average number of eggs contained in each and through that figure to reach the total egg deposition. Counts were made on the following females: 1930—98, 1932—73, 1934—165, and 1936—91. That these counts were made on a sample of females chosen at random was definitely confirmed when it was found that the average weights and lengths showed no significant difference from the average weights and lengths of all the females sampled in the same year.

By the product-moment method described by Pearl (1930) the actual numerical value of the coefficient of correlation between the number of eggs per female and each of the weight in pounds and the length in inches was calculated for each of the four years. In 1932 the weight relation is omitted due to the fact that no weighings were carried out on the females sampled for the counts. The results are as follows:

Correlation coefficient between number of eggs per female and

Year	Total length (inches)	Value of P	Total weight (pounds)	Value of P
1930	0.33	< 0.01	0.36	< 0.01
1932	0.24	< 0.05		
1934	0.35	< 0.01	0.36	< 0.01
1936	0.34	< 0.01	0.38	< 0.01

Using table VA (Fisher 1932) for testing these coefficients, it is found that in every case except 1932 they are definitely significant, P being less than 0.01. In 1932, the coefficient is probably significant since P is less than 0.05 but greater than 0.01. In a given year, therefore, the number of eggs contained in a female increases directly with rise in length and weight.

That this relationship may not hold strictly for the averages for different years can be seen from the sizes and egg counts submitted below:

Year	Average length (cm.)	Average weight (kg.)	Average number of eggs
1930	51.1 ± 0.137	1.64 ± 0.015	1535 ± 12
1932	54.0 ± 0.132	2.04 ± 0.045	1758 ± 15
1934	53.0 ± 0.117	1.76 ± 0.013	1799 ± 11
1936	53.0 ± 0.111	1.70 ± 0.015	1899 ± 12

The number of eggs for the smallest females, those in 1930, are fewest but the count for the largest females, those in 1932, is not the largest, as might be

expected, but intermediate. It is evident, therefore, that the same equation is not obeyed each year. Although the data submitted above are sufficiently complete to permit recognition of the interrelationship for individuals within a given season, they will not allow the calculation of a general equation to cover the four cycles.

SUMMARY

The runs of adult pink salmon to McClinton creek, Masset inlet, B.C., vary little in point of time of appearance of the first fish and the disappearance of the last from year to year. The period occupied for the main portion of the run to reach the spawning beds differs and is dependent chiefly upon the rainfall and freshet conditions.

The proportion of the two sexes did not differ from 1 : 1 in two seasons. In one season the males predominated slightly and in another the females.

It is usual for the males to appear in greater numbers at the first of the run, but a subsequent increased influx of females produces, on the spawning beds, approximate equality between the two sexes.

The average length of males is definitely and consistently greater than that of the females in the same year. The averages for each sex vary significantly from year to year apparently inversely to the total number of McClinton creek pinks in the sea. There is usually a significant increase in length from the beginning to the end of the run.

The average weights of the males are significantly greater than those of the females for the same year. For a given sex they vary consistently from year to year in the same manner as did the average lengths. An increase in weight with the progression of the run is suggested in some cases. The reason that it is not always evident may be due to the fact that there is a loss of weight because the fish are not feeding on the spawning migration.

The number of eggs contained in female pink salmon in any given year increases with increase in weight and length. The average egg content in different years is however correlated much less closely, if at all, with average length or weight.

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Sexual Dimorphism in Vertebral Number in the Capelin *Mallotus villosus* (Muller)

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ABSTRACT

Vertebral counts of capelin spawning in autumn on Vancouver Island were: males 65.65, females 65.13. Differing significantly, these demonstrate sexual dimorphism of a type rare among fishes. The sexual difference is not primarily associated with length, although there is some relationship between length and vertebral number among females. The high degree of sexual specialization in the fins is limited to size and integument and does not include meristic features.

At spawning time the capelin shows a degree of sexual dimorphism unusual among isospondylous fishes. Most noticeable are the four rows of modified scales on the males, two on either side, which produce the villous ridges from which the specific name is derived. Males are distinctly larger than females—some five per cent longer on the average in our material—and much more robust. Other differences between the sexes are to be found in the fins. These are larger in the males, especially those on the under surface of the body, and most noticeably the pectorals and anals. These fins in the spawning male are otherwise modified. On the anterior surface of the pectoral fins the rays bear membranous ridges and on the posterior the rays are lined with tubercles. The first ten to twelve rays of the male anal fin are much enlarged, a condition which is most noticeable approximately between rays seven to ten, which are so enlarged at the distal ends as to practically eliminate the intervening membrane. The males among spawning fish are distinguished also by having tubercles on the lower part of the head including snout, jaw, lower part of the operculum, branchiostegals, and isthmus.

Vertebral counts reported in the present paper show that sexual dimorphism has extended to vertebral number, the male capelin having on the average approximately one-half a vertebra more than the female. An exploratory investigation of fin ray number gave no evidence of sexual dimorphism in other meristic characters.

Sexual differences in meristic character in general are rare among fishes. Hubbs (1918) in referring to a sexual difference in anal fin ray number in certain species of viviparous perches says, "But one other instance of this kind in the whole class of fishes has come to the writer's attention; it is that of the South American Poeciloid genus Cynolebias. Regan . . . . 1912 . . . ."

Vertebral count has been used so generally in the study of fish populations that sexual differences in that character, if present, should certainly have been

J. Biol. Bd. Can. 3 (5) 1937.

recorded and taken into consideration in analysing the data. This has not been done by most investigators. On the other hand, Schaefer (1936) records the absence of any significant sexual difference in vertebral number for surf smelt, *Hypomesus pretiosus*; Tester (1937) states that there is no sexual difference for Pacific herring, *Clupea pallasii*; and the writer obtained negative results on analysing data for some 3,000 vertebral counts for pilchard, *Sardinops caerulea*.

In two species evidence of sexual dimorphism in vertebral number is available in the literature. Hubbs (1925) found the males of Pacific anchovy, *Engraulis mordax mordax* to have a slightly higher vertebral number (45.77) than the females (45.65). This he points out to be "of probable but hardly certain significance" (P c. 0.02). Punnett (1904) working on the elasmobranch *Spinax niger* found the number of "whole vertebrae" to be significantly smaller among adult males (44.62) than among adult females (45.06) (P much less than 0.01) and the number of total vertebral segments also to be smaller among the males (65.04) than among the females (65.47) (P much less than 0.01).

MATERIALS AND METHODS

The material used in making vertebral counts was collected about Vancouver island with beach seines in four years as follows: Departure bay, October ?, 1930; October 5, 1932; September 28, 1934; Hammond bay, October 6, 1936. The fish are readily taken only on the spawning grounds and the numbers of the sexes there are so unequal (333 males to 82 females in 1936) that samples for several years had to be examined in order to have sufficient females for a satisfactory analysis.

After being preserved in formalin the vertebral column was exposed by realizarin (Tåning) 1927) to facilitate accurate counting. The counts were made moving the flesh from one side of the body and the specimens were stained with under the low power of a binocular microscope. In making these counts the terminal ossicle (Ford 1928) (the hypural of many authors) was not included.

Lengths were determined on a measuring board, the caudal end point being the end of the silvery area.

In measuring paired fins, the distance from the insertion to the end of longest ray was taken; for unpaired fins the height recorded was the length of the longest ray. The lengths and heights of fins have been expressed in parts per thousand of the length. All formed rays were counted in paired fins; in unpaired fins only those anterior rays were counted which were half as long as the longest ray and those posterior rays which rose from a separate pterygiophore.

RESULTS AND DISCUSSION

The results of the vertebral counts are shown in table I. For each group of counts is given the sum of the squares of the deviation of the counts from their mean ($S(x-\bar{x})^2$ of Fisher 1932) from which all other constants may be calculated, and the standard error of the mean (σM).

TABLE I. Vertebral number in capelin

Year	Sex	63	64	65	66	67	68	69	70	Males Av.	Males Sum of squares	Males No.	Males σ_M	Females Av.	Females Sum of squares	Females No.	Females σ_M
1930	♂	2	5	30	44	12	5			65.76	94.1	98	0.10				
	♀	2	7	21	7	3								65.05	33.9	40	0.15
1932	♂		2	12	15	1				65.50	13.5	30	0.12				
	♀	2	3	4	5			1						65.13	31.7	15	0.39
1934	♂	1	15	88	73	24	5	1		65.59	175.9	207	0.064				
	♀	1	8	13	4	7								65.24	42.1	33	0.22
1936	♂		7	41	41	13	4		1	65.72	105.6	107	0.10				
	♀	3	18	32	20	5	1							65.11	77.1	79	0.11
All years	♂	3	29	171	173	50	14	1	1	65.65	392.0	442	0.045				
	♀	8	36	70	36	15	1	1						65.13	186.4	167	0.082

From the table it may be calculated that the difference (0.52 excess for males) between the numbers of vertebrae of all the males and all the females is very highly significant. (P much less than 0.01). For the separate years 1930 and 1936 highly significant differences between the sexes are indicated. (P less than 0.01). The differences for the smaller 1932 and 1934 samples were not significant. However, they are in the same direction, and when the counts for these two years are combined a difference of probable significance (P c. 0.016) is indicated. Moreover, the lowest vertebral average for males is higher than the highest for females by a quarter of a vertebra. It appears, accordingly, that the difference in vertebral number between the sexes is a regular occurrence and is independent of year class variation.

There are indications of a relationship between length and vertebral number similar to that recorded by Tester (1937) for herring. (For female capelin less than 97 mm. length, average vertebral number, 64.71; number of specimens, 34; sum of squares, 21: for those more than 96 mm. length, average vertebral number, 65.23; number of specimens, 133; sum of squares, 158: probability of such a difference occurring by chance, 0.01). However, the arrangement of the data as in table II indicates that length is not the principal factor in producing the difference between the sexes.

TABLE II. Average vertebral numbers of capelin in each length group. The length distribution is given by the numbers in brackets indicating the number of counts entering into each average.

Sex and year.	Length in millimetres. - 96	97 - 100	101 - 103	104 - 106	107 - 109	110 -
Males						
1930		65.83(6)	65.46(24)	65.76(42)	66.00(23)	66.0 (3)
1932		66 (1)	65.43(7)	65.60(10)	65.50(8)	65.25(4)
1934		65.56(9)	65.80(25)	65.54(93)	65.49(59)	65.90(21)
1936	65.7 (3)	66.00(6)	65.71(24)	65.54(35)	65.97(29)	65.50(10)
Females						
1930	64.3 (3)	65.05(19)	65.31(13)	65.00(4)	64 (1)	
1932	63.5 (2)	65.38(8)	65.5 (2)	65.3 (3)		
1934	64.86(7)	65.08(13)	65.75(8)	65.40(5)		
1936	64.82(22)	65.13(31)	65.33(18)	65.0 (5)	65.0 (2)	65 (1)

Averages for the proportional fin lengths of ten males and eleven females are: pectoral, males 186, females 133; ventrals, males 164, females 141; anal, males 90, females 55; dorsal, males 127, females 122 (nine only). In spite of the strongly marked sexual dimorphism in the size of fins on the ventral surface of the body there is no evident difference in meristic features, as is shown by the following ray counts on the same material: pectoral, males 17.8, females 17.8; ventrals, males 8, females 8; anal, males 20.2, females 20.2; dorsal, males 12.3, females 12.4. These counts agree with those given for fish taken in northern Europe by Schnakenbeck (1931) for the dorsal (10-13) and pectoral (15-20) fins, but disagree with those of Sleggs (1933) for the dorsal (13-15) for Newfoundland capelin. On the other hand, the anal ray counts agree with that of Sleggs (19-22) rather than those of the former author.

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The Relation of Temperature to the Seaward Migration of Young Sockeye Salmon (*Oncorhynchus nerka*)

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ABSTRACT

The period of seaward migration of young sockeye from Cultus lake each spring is inversely correlated with temperature conditions prevailing during the months immediately preceding. Correlating temperature readings of the outflow stream with date when twenty per cent migration occurred, a statistically significant correlation of −0.85 was obtained for February and March and −0.77 for January to March. Using air temperatures, coefficients of −0.91 for January to March and −0.74 for February and March were found. Commencement of migration coincides with vernal rise in lake temperatures. Under normal conditions, with low winter minimum, there appears to be a threshold migration temperature approximating 4.4°C. (40°F.) but in seasons when lake temperatures are not depressed to this level a slight rise stimulates migration. Progress of migration is largely influenced by prevailing weather conditons and their effect upon temperature trends. Cessation of migration appears to be related to the setting-up of a "temperature blanket" which inhibits migration from the lake of sockeye still resident therein. These latter, showing small growth during their first year, remain in the lake until the following spring and then are among the first to migrate.

During the past eleven years, 1926 to 1936, in conjunction with an investigation of the propagation of sockeye salmon at Cultus lake, British Columbia, (Foerster 1929a, 1934, 1936) studies have been made of the seaward migration of this species and of the factors related thereto. From daily counts of migrating individuals the characteristics of the migration each year—date of commencement, period of maximum run, date of termination—have been revealed. The present paper deals with the relation between these and temperature conditions.

It is characteristic of sockeye salmon that they spawn in the fall of the year, September to December, and normally in streams above lakes, though in some localities such as Cultus lake they select gravel beaches about the lake through which seepage water flows. The fry upon emergence from the gravel of the spawning nest in March or April pass rapidly to the deeper waters of the lake, there to remain until the following year, March to June, when they enter upon their seaward migration. A small proportion, probably produced from spawnings in or immediately adjacent to the outlet stream from the lake, may be found migrating as fry, while a further proportion, varying in numbers from year to year, may remain in the lake a second season, passing to sea as two-year-olds. Very rarely are three-year migrants encountered.

METHODS

Counting of Migrants

By means of a specially-constructed fine-mesh screen counting fence (figure 1), described in Foerster (1929b), erected in the outlet stream, Sweltzer creek,

from Cultus lake, young salmon migrating from the lake could be trapped and retained for counting. The counts represent actual enumeration of all individuals collected daily in the retaining pens, except for the migration of 1927 when the numbers were estimated by weighing all migrants and computing the total number from periodic weighings of known numbers. The migrations occurred chiefly at night and the fish were normally held until the following day for counting. On rare occasions, however, when the down-stream migrants tended to tax the accommodation of the retaining pens, they had to be counted and released during the night. The counts plotted in the accompanying graphs represent, therefore, unless otherwise noted, migrations occurring the previous night.

Water Temperatures

In the summer of 1928, a Julien P. Fries and Sons air and water thermograph was installed at the counting fence in Sweltzer creek and thereafter continuous

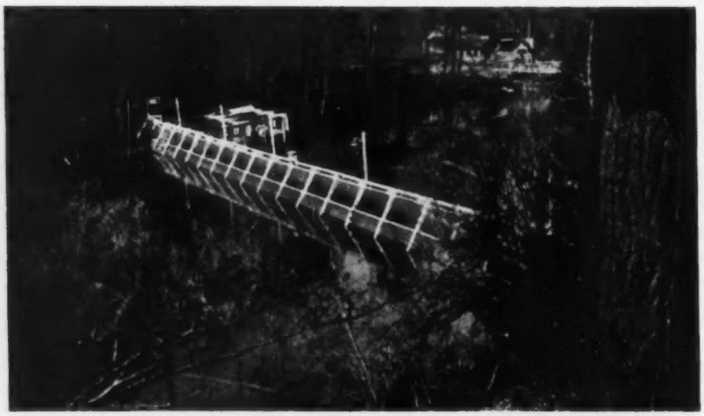

FIGURE 1. A general view of the fine-mesh screen counting fence constructed in Sweltzer creek approximately 200 yards (ca. 185 m.) below the outlet of Cultus lake, by means of which the number of young sockeye salmon migrating seaward from the lake each year is determined.

air and water temperatures, in degrees Fahrenheit, were available. For this paper the Fahrenheit readings have been converted to Centigrade. The water temperature records are those of the outlet creek water since the bulb of the thermograph was placed in the current flowing over the counting fence foundation, but they are interpreted generally as indicating the temperature trends in the epilimnial waters of the lake. Each daily record represents the mean of twelve two-hourly readings.

Air Temperatures

Two sets of air temperature data have been used. For general seasonal temperatures, as in table I, the records of the Dominion Meteorological Service

recording station at Chilliwack, nine miles (14.5 km.) from Cultus lake, have been utilized. They have been taken from "Climate of British Columbia", issued by the Provincial Department of Agriculture each year. For figure 3 the thermograph records for Sweltzer creek have been used and the daily temperatures recorded represent the mean of twelve two-hourly readings.

Other Meteorological Data

The precipitation records are those given in the Monthly Record of Meteorological Observations, issued by the Meteorological Service of Canada for the Chilliwack recording station, 1926 to 1931, and for the Cultus lake hatchery, 1932 to 1936. Hours of sunshine, as recorded at Agassiz, approximately fifteen miles (24 km.) north of Cultus lake, have been provided directly by the Superintendent of the British Columbia Division of the Meteorological Service of Canada at Victoria.

Correlations

In calculating correlation coefficients between temperature and migration of young sockeye the product-moment method outlined by Tippett (1931) has been adopted. The significance of the coefficient was read from Fisher's (1932) table V.A. and is expressed by "P", the probability that the observed correlation is accidental and not indicative of any causal relationship. For temperature the mean yearly (April to March of the following year) and two early spring, viz., January to March and February and March, temperatures have been computed from the mean monthly temperatures. The migration index is represented by the number of days from January 1 of each year to the date when twenty per cent of the migration had occurred. Similar calculations were originally made also for the fifty per cent stage of each run but as the data so obtained followed the same trend as those for twenty per cent they have not been included.

CORRELATION BETWEEN TEMPERATURE AND PERIOD OF MIGRATION

In figure 2 are plotted the accumulated percentages of each year's migration from 1926 to 1936. The seasonal occurrence and duration of each run, from the day when the first migrants were observed to that on which the last were counted, are indicated, and, in addition, that interval during which from twenty to eighty per cent of the migration took place has been blocked in to provide a comparable index of the "relative period of migration". For each year the extent of such period is shown and the variability from year to year is readily apparent.

For correlation computations, as stated in the previous section, the date when twenty per cent of the migrants had been counted each year was ascertained and the numbers of days these dates represent from January 1 were taken to provide a comparable index of the time of the migration. These may be set down as:

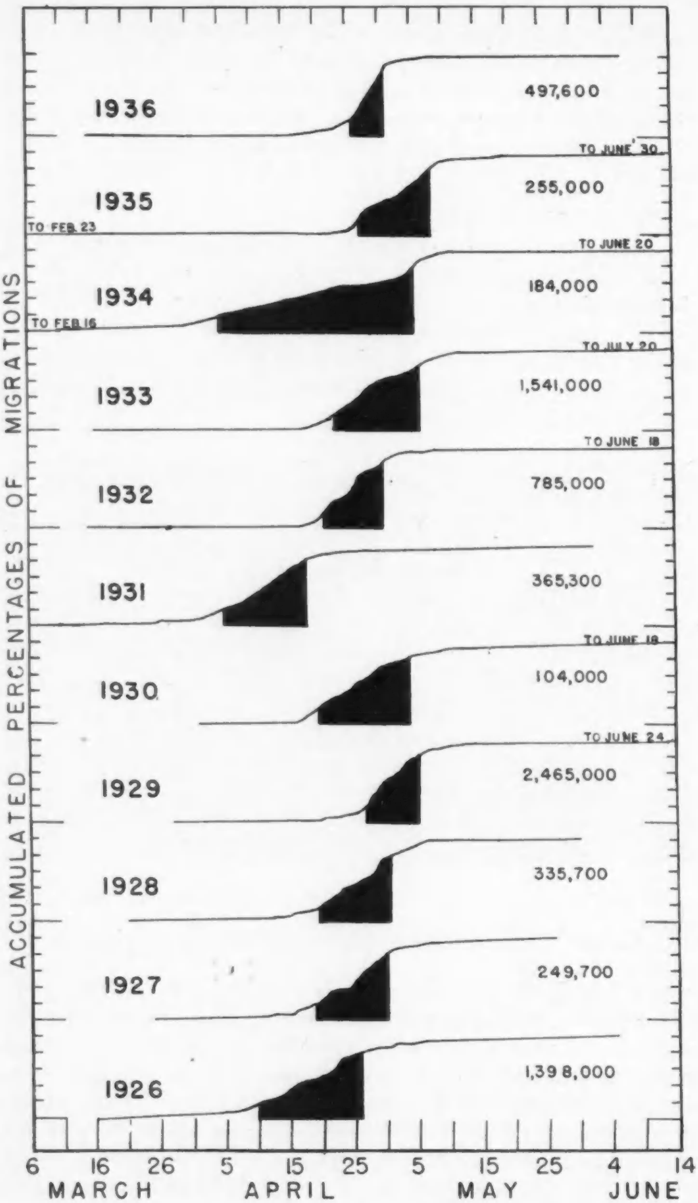

FIGURE 2. The seaward migration of young sockeye salmon from Cultus lake each year from 1926 to 1936 plotted as accumulated percentage curves. The dates of commencement and termination of each season's run are given. The period from the date when twenty per cent of the run occurred to that with eighty per cent, is blocked out. The total numbers of migrants counted each year are given at the right of the figure.

Year	Date of 20% of the run	Number of days from January 1
1926	April 10	100
1927	" 19	109
1928	" 20	111
1929	" 27	117
1930	" 20	110
1931	" 5	95
1932	" 21	112
1933	" 22	112
1934	" 5	95
1935	" 27	117
1936	" 26	117

The air and water temperature data, obtained as indicated in the preceding section, are shown in table I. The yearly air temperatures have been arranged from April to March of the following year to correspond with the approximate period of residence of young sockeye in the lake. Water temperatures are available only from 1929 and only those for the early spring months, January to March, are tabulated. Mean yearly water temperatures for 1929 and thereafter were computed but are not here tabulated.

From the two sets of data thus derived, temperature on the one hand and period of migration on the other, the following correlation coefficients and their significances have been obtained:

Time of migration and	Number of years	Correlation coefficient	P.
Mean yearly air temperature	11	−0.55	0.1 to 0.05
Mean yearly water temperature	8	−0.12	0.8 to 0.7
Jan. to Mar. air temperature	11	−0.91	less than 0.01
Jan. to Mar. water temperature	8	−0.77	0.05 to 0.02
Feb. and Mar. air temperature	11	−0.74	less than 0.01
Feb. and Mar. water temperature	8	−0.85	less than 0.01

As noted above, young Cultus lake sockeye salmon spend one year, normally, in the lake before migrating seaward. During this time they appear to inhabit chiefly the deeper strata of the lake and subsist largely on plankton (Foerster 1925; Ricker 1934). Their development, therefore, is intimately related to limnological conditions in the lake waters, as these influence the seasonal abundance and distribution of plankton crops and affect the rate of metabolism of the sockeye themselves. With the arrival of spring and the consequent gradual change in lake conditions from winter stratification to a spring overturn and subsequently to a setting-up of summer stratification, the sockeye depart from the lake.

Since from the plotted progress of the seaward migration each year, figure 2, definite variations in the time of the run are revealed, the question arises—are the variations related to the general environmental conditions experienced

by the sockeye in the lake throughout their period of residence therein as affecting their general development? Or are they due to the varying nature of the onset of spring conditions in the lake as these bring about the vernal limnological

TABLE I. Mean air and water temperature data for Cultus lake. The air temperatures are from reports of the Dominion Meteorological Service and converted into degrees Centigrade. Water temperatures are from thermograph readings for Sweltzer creek.

Air temperatures	1925 1926	1926 1927	1927 1928	1928 1929	1929 1930	1930 1931	1931 1932	1932 1933	1933 1934	1934 1935	1935 1936
April	10.6	12.8	8.3	9.5	7.8	11.1	11.1	11.1	10.0	13.3	9.5
May	15.0	13.3	11.7	11.1	13.3	12.2	15.0	13.3	11.7	14.4	12.8
June	16.1	17.2	17.2	15.6	15.6	15.6	15.6	17.2	15.0	17.2	15.0
July	18.9	18.9	18.9	17.8	17.2	17.8	18.3	16.7	17.2	16.7	17.8
August	16.7	17.8	18.3	17.2	17.8	17.8	17.2	17.8	18.3	18.3	17.8
September	14.4	12.8	14.4	13.9	15.0	14.4	14.4	14.4	12.2	13.3	16.7
October	8.3	11.1	10.0	10.0	10.6	8.3	10.0	11.1	8.9	10.6	9.5
November	5.0	7.2	3.9	5.0	4.4	4.4	3.3	7.2	6.1	7.8	4.4
December	6.1	1.1	-2.2	2.2	0.6	3.9	2.8	1.7	1.1	1.7	6.1
January	3.3	0.6	1.1	-2.8	-4.4	5.0	0.6	3.3	4.4	-1.1	2.8
February	3.9	3.9	3.3	0.0	4.4	4.4	2.2	1.1	6.7	6.1	-3.3
March	9.5	5.6	5.0	6.7	7.2	7.2	6.7	6.7	9.5	4.4	3.3
Mean for 12 months	10.7	10.2	9.2	8.9	9.1	10.2	9.8	10.1	10.1	10.2	9.4
Mean for Jan. to Mar.	5.6	3.4	3.1	1.3	2.4	5.5	3.2	3.7	6.9	3.1	0.9

Water temperatures	1929	1930	1931	1932	1933	1934	1935	1936
January	4.7	2.7	5.7	5.7	4.6	5.1	4.4	5.4
February	2.6	1.8	5.3	3.4	3.0	5.0	3.6	2.4
March	3.9	3.1	5.9	4.2	3.8	6.4	4.2	2.7
Mean for 3 months	3.7	2.6	5.6	4.4	3.8	5.5	4.1	3.5
Meah for Feb. and Mar.	3.3	2.5	5.6	3.8	3.4	5.7	3.9	2.6

changes? To arrive at an answer the correlation coefficients listed above may be reviewed.

Water temperatures would naturally be expected to be more closely related to the reactions of sockeye than those for air and have been so considered. Air

records have been studied, however, because of the relationship in certain instances existing through the close correlation of air and water temperatures and also because, where significant correlations may prevail, the air temperature data are much more readily and more widely obtained.

With respect to the first phase of the question mentioned above, the relation of time of migration to the lake limnological conditions prevailing during the whole of their period of residence in Cultus lake, it is evident, from the correlations, that no significant relationship exists. For the water temperature data for Sweltzer creek the correlation coefficient is only −0.12 (P. between 0.8 and 0.7). These data which are assumed to indicate the temperature conditions in the epilimnial stratum of the lake are therefore of little value in explaining the variation in migration time. For the mean yearly air temperatures the correlation coefficient is −0.55 (P. 0.084), a doubtfully significant relationship. Furthermore the correlation coefficient calculated for the air and water temperatures for each yearly period from 1927 to 1936, 0.36 (P. 0.38), clearly signifies that the mean yearly air temperatures do not reflect the variations in mean yearly surface water temperatures of the lake.

Turning, then, to the alternative possibility, namely, that the migrations are influenced primarily by the temperature conditions prevailing during the months immediately preceding the seaward movement, correlation coefficients have been calculated for two periods, January to March, and February and March. For both periods very close relationships occur. Whether air or water temperatures are utilized, significant coefficients are obtained. Moreover they are invariably inverse, thus demonstrating that the lower the temperature, i. e., the colder the weather during these months, the later the migration.

Correlation coefficients computed for the mean air and water temperatures for each early spring period are as follows: January to March, 0.82 (P. between 0.02 and 0.01), and February and March, 0.69 (P. 0.04). These data indicate that for the purpose of establishing the influence of January to March temperature conditions upon the time of migration of young sockeye in an open lake such as Cultus lake, which is but rarely frozen over and then but lightly for a short period, the air temperatures may be used as an alternative to water temperatures.

Though size of migrants is shown below to have a distinct influence upon period of migration in certain years, no pronounced relationship was found to exist between size of yearling migrants and time of 20 per cent migration in the series of years under review. Calculation of the correlation between these two factors provided a coefficient of −0.22 (P. between 0.6 and 0.5).

From the data obtained, therefore, it is shown that the seaward migration of young sockeye in the spring is definitely influenced by the temperature conditions prevailing during the months of January, February and March preceding the migratory movement and in inverse relationship. While the migration from Cultus lake normally occurs toward the end of April its relative occurrence is advanced or delayed by the mildness or severity, respectively, of the climatic conditions during the period from January to March. The correlated trends, represented by the regression lines as calculated from the correlation coefficients,

for both air and water temperatures and the number of days from January 1 to the date when twenty per cent of the run occurs, are indicated by the equations:

(January to March mean air temperature) $= 25.4 - 0.20$ (days to 20% of migration)

(February to March mean water temperature) $= 15.85 - 0.11$ (days to 20% of migration)

indicating, respectively, that (1) when the mean January to March air temperature is 2°C., twenty per cent of the migration will occur 117 days from January 1 or April 27-28, and with every 1°C. increase in the mean temperature the twenty per cent stage of migration will occur five days earlier and (2) when the mean February and March water temperature is 2°C. twenty per cent of the migration will have occurred 126 days from January 1 or by May 6 and with each 1°C. increase in the mean water temperature the period of migration will occur nine days earlier.

The equation for the regression line of January to March mean air temperatures on February and March mean water temperatures, the correlation coefficient of which is 0.91 (P. less than 0.01), has been computed as:

(January to March mean air temperature) $= 1.512$ (February and March mean water temperature) $- 2.42$,

showing that when the mean Jeanuary to March air temperature is 2°C. the mean water temperature for February and March will approximate 2.92°C. and that for each increase of 1°C. in mean air temperature the water temperature will increase approximately 0.66°C.

A definite inverse relationship having been shown to exist between the time of general migration of young sockeye from Cultus lake and the early spring temperature conditions the remaining sections of the paper are devoted to a study of particular phases of the migration and the possible influencing factors.

THE COMMENCEMENT OF THE SEAWARD MIGRATION

In the diagrammatic portrayal of each season's seaward migration from 1926 to 1936, figure 2, the dates when the first migrants appeared are indicated and the long periods during which the daily counts were comparatively small may be noted. Although the earliest migrants are very likely individuals, who have, by reason of their particular location in the lake, been more readily stimulated by the influences causing migration than the general body of sockeye in the lake, an effort has been made to deduce from the environmental conditions prevailing at the inception of migration those factors which activate the seaward movement. These must early appear and gradually extend to the mass of sockeye residing in the deeper waters of the lake.

Chamberlain (1907), from studies of the movements of Alaska red salmon in Karluk lake and a comparison of his results with conditions reported by Babcock (1904) for the Thompson and Fraser rivers, British Columbia, at the time of the migration of young sockeye, concluded "that migration begins when the temperature of the water has reached approximately 40°F.", or about 4.4°C. These observations apply essentially to that type of lake which freezes over

during the winter and in which as and after the ice disappears there occurs an appreciable increase in the temperature. Cultus lake, on the other hand, presents an entirely different set of conditions, because of its location in a mild coastal temperate area, whereby it but seldom freezes, and then but thinly and for a short period. Nevertheless the data obtained pertaining to the onset of migration and water temperature generally substantiate the conclusions of Chamberlain.

From the temperature readings obtained when the first migrants appeared it was found that in the years 1929, 1930, 1932, 1933 and 1935 the water temperatures varied around 4.0°C. to 4.4°C. Bearing in mind the fact that they represent stream-water readings and hence may not quite accurately reflect the lake temperature conditions, it is seen that in these years, when the seasonal conditions were ostensibly normal, i.e., low temperatures in late January and during February succeeded by a gradual rise in March, the sockeye resident in the lake did display a tendency to migrate seaward when the water temperatures approached 4.4°C. or 40°F. Two notable exceptions occurred, however, namely when, as in 1931 and 1934, the water temperatures did not drop below 5.0°C., and when, as in 1936, a pronounced and extended period of cold weather depressed the water temperatures to below 4.4°C. until well on in April. The trends of the late winter and early spring water temperatures can be observed from the mean monthly temperatures given in table I above.

The coincidence of the commencement of migration with a water temperature of 4.4°C. appears, however, when the unusual conditions of the years 1931, 1934 and 1936 are studied, to be primarily caused by the regular progression of limnological conditions in the lake. Temperature data obtained by Dr. W. E. Ricker in the years 1932 to 1936 at regular intervals for the different strata of Cultus lake (Ricker 1937, p. 370) show that each year a decrease in lake water temperatures occurs to a minimum low, the period of this latter varying appreciably each season but usually being found in late January or in February. Thereafter a gradual rise in temperature takes place.

Though the exact date on which the lowest limit of temperature was reached is not revealed by the data, it is noted that in the years 1932, 1933 and 1935 the dates at which the first migrants appeared, March 15, March 16 and February 23, respectively, coincide with a noticeable increase in the temperature and, incidentally, with an increase to approximately 4.0°C., particularly in the upper waters of the lake. Similarly in 1934, though the minimum temperature did not apparently drop below 5.0°C. a slight increase occurred by February 14 and two days later the first migrants appeared. Again in 1936, when the water temperatures remained unusually low, with a uniform minimum of around 2.5°C. to 2.8°C. on March 2, an increase to 3.2°C. is noted by March 16. During the night of March 14 the first migrants of the year left the lake.

Studying more closely this apparent correlation existing between onset of migration and the vernal rise in water temperature, the data have been arranged to show the amount of temperature increase occurring up to the time when certain definite stages of the migration have occurred. As noted elsewhere above, the temperatures of Sweltzer creek at the counting fence site are considered as indicating the temperature trends in the epilimnial strata of the lake

above, and in table II there are shown for the years 1929 to 1936 the mean winter minimum temperature and the mean temperatures prevailing on the days when certain stated percentages of migration had been counted. In table III are given the temperatures for Cultus lake at surface and at 5 metres, interpolated from the readings obtained by Ricker (1937, tables II and III) for the years 1932 to 1936.

TABLE II. Temperature readings in degrees Centigrade of Sweltzer creek, showing the mean winter minimum (usually occurring in Febraury) and the mean daily temperature prevailing on the days when stated percentages of the migration had been counted. The amount of increase in temperature can be readily determined by subtracting, for each year, the winter minimum.

	1929	1930	1931	1932	1933	1934	1935	1936
Minimum winter temperature °C.	2.0	1.1	4.8	2.5	2.2	4.5	2.8	1.7
Temperatures when following percentages of migration were counted:								
0.05	5.3	4.6	5.4	5.1	5.5	4.6	4.6	3.8
0.1	5.2	4.4	5.4	5.4	6.1	5.1	4.8	4.0
0.5	5.5	5.2	5.8	5.1	6.5	4.8	6.0	4.9
1.0	6.6	4.6	6.2	5.2	6.0	5.4	8.1	4.3
5.0	8.8	6.2	5.9	6.8	6.9	6.5	8.8	6.3
10.0	9.3	6.2	6.4	6.9	8.4	7.7	8.0	8.1
20.0	8.4	6.6	7.0	7.9	8.9	10.1	6.7	9.9

In attempting to relate the movements of sockeye to temperature changes in Cultus lake the chief obstacle is the lack of knowledge as to the depth in the lake at which the sockeye are moving immediately prior to migration. There is some evidence to indicate at this time, when lake temperatures are relatively uniform throughout, the fish are more or less generally distributed but further information is necessary. In any event the data of tables II and III indicate: (1) Commencement of seaward migration—when 0.05 per cent of the run had been counted—occurred when a definite increase in lake temperatures had taken place. In six years out of the eight for which mean temperatures of Sweltzer creek are available, an increase of 2.8 ± 0.22°C. in temperature took place to the 0.05 per cent stage of migration. In the remaining two years, 1931 and 1934, when increases of only 0.6°C. and 0.1°C., respectively, were obtained, the minimum winter temperatures were unusually high. Comparison of lake water temperatures at 5 metres depth shows that for 1932, 1933, 1935 and 1936, when minimum winter temperatures were low (average—3.2°) an average increase of 1.56° occurred while in 1934, with a high winter minimum of 5.4°, the increase was only 0.2°. Lake surface water temperatures show average increases of 2.2° for the four years of low winter minima and 0.3° for 1934. The consistent upward progressions of the temperatures are highly indicative of a regular vernal trend at the time of onset of migration. (2) When migration definitely gets under way the temperatures of the upper strata of the lake, as indicated by the Sweltzer creek readings and those of the lake at 5 metres, are in the neighbourhood of

4.9°C. Lake surface temperatures are somewhat higher, the mean for four years being 5.4°, and probably less reliable because of the variable effect of sudden abnormal meteorological changes. (3) A striking correspondence appears between the lake temperatures of each year and the percentage of the migration which has left the lake, quite irrespective of either the time of year at which the percentage migration occurs or the period occupied by the portion of the migration in question, particularly for the years 1932 to 1935. For example, in 1934 from 0.1 per cent to 20 per cent of the migration left the lake during the period from February 15 to April 5, whereas in 1935 they passed out from April 6 to April 27, and in 1933, from April 12 to April 23. The 1936 temperatures are somewhat lower in the 5 metre stratum, but since the surface temperatures show a closer agreement with earlier years, it is probable that temperatures at intermediate depths might correspond more generally. There is evidence of a more rapid warming of the upper waters of Cultus lake in the spring of 1936 from an unusually late winter minimum, and intermediate temperatures between surface and 5 metres might be higher, with the surface temperature on the date of reading being depressed by prevailing meteorological conditions.

TABLE III. Temperatures of Cultus lake at surface (s) and at a depth of 5 metres (5m.) on the dates indicated, at which times certain stated percentages of migration had occurred. The vernal temperature increase may be ascertained by subtracting, for each year, the mean minimum winter temperature.

	1932			1933			1934			1935			1936		
	Date	S.	5m.	Date	S.	5m.	Date	S.	5m.	Date	S.	5m.	Date	S.	5m.
Minimum winter temperatures, °C.	Feb. 16	3.5	3.5	Feb. 17	2.9	3.1	Jan. 15	5.4	5.4	Jan. 25	3.5	3.5	Mar. 2	2.5	2.5
Temperatures when the following percentages of migration were counted:															
0.05	Mar. 29	5.2	4.9	Apr. 10	6.1	5.1	Feb. 10	5.7	5.6	Apr. 3	5.2	4.9	Apr. 8	4.8	4.0
0.1	Apr. 1	5.4	5.1	Apr. 12	6.7	5.3	Feb. 15	5.8	5.6	Apr. 6	5.4	5.1	Apr. 10	5.2	4.2
0.5	Apr. 6	7.6	5.4	Apr. 14	7.2	5.4	Feb. 24	5.7	5.6	Apr. 10	6.9	5.4	Apr. 14	5.8	4.6
1.0	Apr. 9	8.7	6.2	Apr. 15	7.4	5.6	Mar. 4	5.7	5.7	Apr. 12	7.7	5.6	Apr. 15	6.0	4.7
5.0	Apr. 18	[illegible]	7.4	Apr. 20	8.3	6.7	Mar. 23	7.1	6.5	Apr. 24	8.0	7.0	Apr. 18	7.6	5.8
10.0	Apr. 20	8.6	7.5	Apr. 21	8.5	7.0	Mar. 31	8.1	6.9	Apr. 26	8.3	7.4	Apr. 23	9.0	7.0
20.0	Apr. 21	8.6	7.6	Apr. 23	8.8	7.6	Apr. 5	8.8	7.5	Apr. 27	8.7	7.7	Apr. 26	9.9	7.8

From the data here presented it would appear, therefore, that for young sockeye salmon in Cultus lake the factor stimulating migration is essentially one of rising temperatures. When the vernal climatic conditions bring about a rise in lake temperatures, epilimnial strata being first affected, the sockeye commence a general movement in the lake leading to seaward migration. There would appear, however, to be a definite threshold temperature range at which migration commences, for an inverse relationship is shown between the extent of increase required to stimulate migration and the minimum winter temperature. In those years when the minimum lake temperatures were high (4.5° to 4.8°) a much smaller increase was related to a definite percentage of migration than in years

when the winter minima were low. This threshold temperature would appear to be in the neighbourhood of 4.4° to 5.0°C.

That seaward migration is definitely associated with a rise in temperature of the lake waters is indirectly indicated by the fact that in the late autumn when lake temperatures fall to and below the optimum range for migration, as here indicated, no migration of sockeye from the lake occurs. The counting fence has been kept closed for a number of years to check the possibility of autumn migration but none has yet occurred.

Hours of Sunshine and Migration

From the superficial evidence of a cyclic trend in the times of migration of sockeye salmon from Cultus lake, figure 2, in the years investigated it was thought that there might be a definite relation between the time of migration and the amount of sunlight occurring at Cultus lake during the spring months.

Accordingly hours of sunshine records for the lower Fraser valley, as measured at Agassiz, were obtained for each year, commencing January 1. The relationship may be indicated by considering the numbers of hours of sunshine accumulated up to the time of 0.05 per cent of the migration, as follows:

1926—Mar. 17, 200 hours	1927—Apr. 3, 236 hours	1928—Mar. 28, 254 hours
1929—Apr. 10, 273 "	1930—Apr. 2, 275 "	1931—Mar. 10, 145 "
1932—Mar. 29, 170 "	1933—Apr. 10, 233 "	1934—Feb. 10, 191 "
1935—Apr. 3, 215 "	1936—Apr. 8, 223 "	

The seaward migration of young sockeye, therefore, is not directly influenced by the amount of sunshine occurring in the months prior to migration. Once migration has commenced, however, the amount and intensity of the sunlight may have a more or less immediate effect, as shown below.

THE PROGRESS OF MIGRATION

Once the seaward migration of young sockeye has commenced there follows a long interval during which the daily arrivals are relatively small in number. As displayed in figure 2, this period varied exceedingly in extent and, as indicated in the previous section, appears to be definitely related to lake temperatures.

Of particular interest, however, is the speed with which the major portion of the migration, as represented by the twenty to eighty per cent levels (the shaded portions of figure 2), proceeds. It will be observed, for example, that in 1929 the bulk of the 2,465,000 migrants were counted in slightly over ten days, whereas in the following year the bulk of the 104,000 sockeye appeared over a period of fifteen days. Similar comparisons can be made for the years 1933 and 1934.

While evidence has been presented in previous sections to show the effect of temperature upon migration generally and also of limnological conditions upon the commencement of migration, the elucidation of the factors directly affecting the movement of the young sockeye from the lake is much more difficult. The problem involving, as it does, temperature, sunlight, precipitation, wind

action, as these affect the surface waters of the lake and the rate of mixing of the deeper waters, is exceedingly complex, and renders the isolation of particular influences extremely difficult. In figure 3 are presented diagrams of the main portions of the migrations from day to day for each year, together with data concerning prevailing water and air temperatures and precipitation. It will be observed that in the majority of cases the runs rapidly increase with increase in water temperature, but an exception is found in the year 1931 and again to

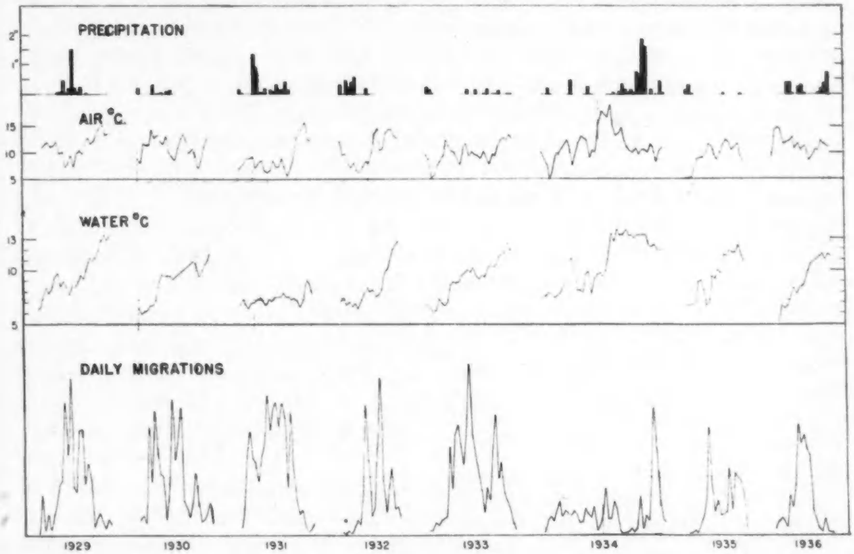

FIGURE 3. Daily counts of migrants, water and air temperatures in °C, Sweltzer creek, and rainfall in inches for the main portions of each year's seaward migration of young sockeye from 1926 to 1936. The migrant counts are not plotted to the same scale throughout and are intended to show the daily fluctuations, rather than actual numbers. The date of commencement of each graph is as follows: 1929—April 19; 1930—April 15; 1931—April 1; 1932—April 13; 1933—April 13; 1934—March 29; 1935—April 20; 1936—April 14. Since the migrants arrived at the counting fence during the night they were normally counted the following day, but in order to present a more accurate comparison with meteorological conditions, the counts when plotted were set forward one day.

a lesser degree in 1934. In these two years, as explained above, the minimum winter temperatures were notably higher than in other seasons.

Prevailing weather conditions play a large part in the daily variations in numbers of migrants departing from the lake. Bright, calm days invariably result in an increase, provided the conditions are not too much prolonged, whereas dull weather, with or without rain, produces a slackening. Wind from the west, i.e., blowing toward the outlet, retards the run presumably by reason of the wave action set up at the lake outlet.

Although no data concerning the strength or scope of influence of surface or sub-surface currents in the lake are available, it would seem that the young

sockeye, attracted to the surface strata of the lake by the warming of the waters, are definitely influenced by the currents there occurring. As previously mentioned, on calm evenings following a bright, warm day, the surface waters of the lake adjacent to the outlet are literally alive with young sockeye jumping from or finning the surface and the migrations after dark are large. Further data regarding the relation of lake currents to wind action and sockeye migration are being obtained.

The progression of limnological conditions affecting the seaward migration of young sockeye may thus be postulated: (1) The warming of the waters of the upper strata of the lake attract the sockeye into them. Bright, warm weather increases such movement and may be appreciably aided by the action of the winds in producing more rapid mixing of the waters. On the other hand, heavy precipitation and dull, cool weather inhibit the reaction. (2) In the surface water the sockeye respond to the stimulus of the currents in the lake and are directly influenced by the strength and character of these currents as they vary with the prevailing climatic conditions.

CESSATION OF MIGRATION

From the diagrams in figure 2 it is observed that the closing days of each year's migration are marked by an extended period of daily decreasing numbers of sockeye issuing from the lake. The numbers of stragglers involved and the periods over which they occur vary from year to year, and seem to be but indefinitely related to the water temperatures of Sweltzer creek as these are shown for the closing date.

Were it to be concluded that these stragglers appearing at the close of the migration period represented the last of the young sockeye in the lake, the trend of the latter phase of the run would appear to be natural. Observations and counts indicate, however, that quite frequently there continues to linger in the lake a remnant of the population and that these extend their residence until the following spring. Chamberlain (*loc. cit.*) has remarked upon this remarkable feature and it has been indicated for Cultus lake in previous papers (Foerster 1929b, 1934, 1936). The occurrences for Cultus lake, as determined from migration counts may be summarized as follows:

Year	Yearlings	Two-year-olds
1926	1,398,000	not determined
1927	183,200	66,700
1928	334,000	1,700
1929	2,457,000	8,000
1930	38,000	66,000
1931	350,000	5,100
1932	785,000	200
1933	1,541,000	none observed
1934	121,000	63,000
1935	241,000	14,000
1936	496,000	1,400

While these counts of the two age classes are based on random sampling of the migrations and represent, therefore, only the most probable numbers, they are sufficiently accurate to display the variation from year to year in the number of two-year individuals and more particularly the increase in the two-year group in the years following a very large migration.

From a study of the growth-rates of the two-year-old seaward migrating sockeye, as computed from the scales, it has been found (Foerster 1929b, 1934, 1936) that these individuals are invariably of smaller size at the end of their first year than are yearlings which migrate at that time. It has further been shown from a study of the migrants of 1928 (Foerster 1936), confirmed by findings obtained for the 1929 migration (now in manuscript) that when the two-year-olds do migrate they are generally of much larger size than the yearlings of the same migration and also that they appear in the early portion of the season. Moreover the data for the 1929 migration indicate that whereas, as previously determined (Foerster 1929b, 1936) for the relatively small migrations of 1927 and 1928, no significant change in size of migrants occurred during the migration, yet when the migration is large a definite diminution in size of individuals occurs as the migration proceeds. The picture presented by these findings shows, therefore, that size is intimately associated with reaction to the migratory stimulus, and that not only do the large two-year-old individuals respond early but, among the yearlings, the larger react first. At the close of the migratory period there remain only the small yearlings represented by the outgoing stragglers and the still smaller individuals which do not succeed in migrating and which remain in the lake for a second season.

What factors are responsible for the non-migration of these latter it may be extremely difficult to reveal exactly, but the data for Cultus lake point suggestively to a definite temperature influence, similar in creation and in operation to that which Ward (1932) has described in an attempt to explain the origin of the landlocked salmon, namely, a "temperature barrier or blanket" which is created in the epilimnion of the lake by the rapidly rising temperatures of early summer and which effectively inhibits the passage of sockeye through it.

Though the temperature data available (Ricker 1937, tables II and III) are hardly extensive enough to establish a relationship between lake water temperatures in June and the cessation of migration they do conform in 1934 to the temperature blanket idea under discussion. It will be noted that in that year, although the total migration amounted to only 121,000 yearling migrants, a relatively heavy migration of two-year-olds (14,000) occurred in 1935. On June 16, when the migration was practically over, the lake waters in the strata above 10 metres were appreciably higher than in other years at the same period.

The exact temperature level at which the above-mentioned temperature blanket or barrier becomes effective has not been sharply delineated. Ward (*loc. cit.*) reports for Baker lake, Washington, that when the surface water passes 10°C. the sockeye, until then at the surface of the lake, withdraw to the deeper layers and much before surface temperatures of 15°C. are reached the fish are no longer seen. For Karluk lake, Alaska, Chamberlain refers to a diminution in the run when surface temperatures of 50°F. (10°C.) occur. The present data

show that (1) the highest mean daily temperature of the surface water of Cultus lake, as indicated by the thermograph records of Sweltzer creek, when eighty per cent of the migration had occurred, figure 2, was 12.5°C. in 1935 and that generally the mean temperature at this stage of migration was substantially lower—average for eight years being 10.6 ± 0.44°C.; (2) as the surface water temperatures approached 13.0°C. the daily migrations decreased greatly, as shown in figure 3; and (3) thereafter only stragglers appeared until the migration terminated at temperatures ranging from 14.4°C. in 1930 to 20.1°C. in 1933—the average for eight years being 17.5 ± 0.78°C.

It is suggested therefore that the cessation of migration is definitely related to the creation in the epilimnial waters of the lake of a temperature blanket through which yearling sockeye, presumably late in responding to the migration stimulus, are unable to pass. As a result they return to the deeper waters of the lake, there to remain until the migratory influence is again felt the following spring. At this time the two-year-olds are among the first to respond.

Any such theory must, however, take cognizance of the fact that in Cultus lake there are frequently found populations of sockeye which have seemingly lost the migratory reaction and remain in the lake to mature. From his gill-net catches in Cultus lake, Dr. Ricker reports them in small numbers each year and they appear to be largely males maturing precociously in their third year. The exact relationship existing between sea-running sockeye salmon and the land-locked variety (*O. nerka kennerlyi*) and the manner in which the latter have departed from the habits of their progenitors are as yet but imperfectly understood. Chamberlain refers to the possibility of the landlocked form having lost the "primal instinct" to migrate seaward, and of forming a phase of the process of evolution of sockeye from an anadromous to a permanent freshwater type. Ward presents evidence, on the other hand, to show that the land-locking is essentially a process of geographic and climatic change following glaciation, whereby the temperatures of native sockeye-dwelling lakes become so changed that the sockeye are unable to escape.

While Ward's explanation unquestionably presents in admirable fashion the factor responsible for the occurrence of land-locked sockeye in bodies of water now cut off from access from the salt water, it would appear that the same factors which he has enunciated may apply likewise to a more limited extent in areas where runs of sockeye still persist and flourish. It has been indicated above that the setting-up of a temperature blanket in the surface waters of Cultus lake may successfully halt the further migration of those young sockeye, which, because of slow or late development, react late in the spring to the migratory stimulus, and, further, that these individuals appear to respond early to these same migratory influences the following season. Rates of growth of individuals which do not migrate the first or second year and thereafter remain in fresh water to maturity have not been computed but there is a possibility that these fish represent a group which, though not migrating the first year because of retarded development, have experienced extremely good growth in the lake during the second season and thus reached a stage of growth and of approaching maturity wherein the migratory stimulus is not effective. They

do not take part in the seaward migration but remain to mature in the lake precociously in their second or third year. The great majority of these individuals are males.

SUMMARY

The time of the downstream migration of young sockeye salmon from Cultus lake is dependent upon the temperature conditions prevailing during January, February and March immediately preceding the migration. By correlating the mean air and water temperatures of these months with the time when twenty per cent of the seaward migration had occurred, over a period of eight years, statistically significant coefficients of -0.91 for air and -0.77 for water were obtained. The mean water temperatures for February and March provide a correlation of -0.85. The correlations are inverse, indicating that the colder the weather the later the migration, and regression line equations have been calculated to show the generalized relationship.

Although the data generally substantiate previous findings that migration commences when the water temperatures approximate 4.4°C. (40°F.), the records for years in which such minimum temperatures were never reached and for one year when the water temperature remained below this level until late spring, indicate that increase in temperature from the winter minimum appears to be the activating influence leading to migration. Migration seems to be unrelated to any definite degree or spread of increase but definitely associated with a threshold temperature range, lying between 4.5° and 5.5°C.

The speed of migration appears to be closely associated with limnological changes in the lake, which in turn are largely influenced by meteorological or climatic conditions, hours of sunlight, amount of precipitation, frequency and force of the wind, etc. The daily departures from the lake are accelerated by bright, calm days whereas cool, dull weather, with or without rain has a retarding effect.

Evidence is produced to show that not all of the sockeye resident in the lake migrate at the end of their first year therein. When surface waters rise above 10°C. the passage of young sockeye appears to be inhibited and consequently, as summer stratification is set up and the epilimnial waters rapidly increase in temperature, they form a temperature barrier or blanket to terminate the further migration of sockeye. Data disclose that size is an important element in migration and the larger individuals respond most readily. The small yearlings react late and in some cases probably too late to reach the lake outlet before the temperature blanket effectively blocks it.

Many of these latter respond early to migration the following spring but it is suggested that among those experiencing very rapid growth in the lake during their second year's residence the positive reaction to seaward migration is inhibited. They thereupon remain in the lake and mature precociously.

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Studies on Salt Fish

II. The Effect of Salt Concentration on Preservation

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ABSTRACT

The preserving action of salt, as determined chemically and bacteriologically, increases with increasing salt concentration (8 to 32%) and decreasing temperature (21 to 10°C.). It shows in a lengthening of the period of lag before rapid increase in volatile bases. The increase begins when the bacterial count reaches 10-20 million per ml. on 10 per cent salt agar, and its rate is not influenced by salt concentration. The hypothesis is advanced that the trimethylamine oxide present in the fish is reduced very rapidly to trimethylamine when the reduction potential reaches a definite point.

The fishing industry has long depended on salt for lengthy preservation of fish. By varying the amount of salt in relation to the weight of the fish a series of cures is obtained from light salted to heavy salted. By varying the extent of drying, the series is further altered. In most cases the salt concentration of the water in the tissue is made great enough to insure good preservation. In this way the ratio of salt to dry tissue varies with the type of cure. In all cases the success of the cure depends largely on the temperature during the salting and the weather conditions for natural drying. Thus the quality may depend also on the length of time between salting and the end of drying.

The preserving action of salt is generally believed to depend on the increased osmotic pressure, which removes water from the tissues and makes the liquid remaining in the tissues unavailable for most of the bacteria causing decomposition. Some bacterial types have adapted themselves to higher osmotic pressure and can live even in saturated brines. However, very few are proteolytic. Rockwell and Ebertz (1924) also state that part of the preserving action is due to the direct effect of the chloride ion, to the insolubility of oxygen in brine and to the interference with the rapid action of proteolytic enzymes.

This preliminary paper deals with the preserving action of salt at varying concentrations on fish protein material. The changes in certain strata of the bacterial population and in certain chemical products have been followed at two different temperatures.

METHODS

The use of flesh in bacteriological and chemical determinations on fish leads to many difficulties. Apart from errors due to the handling of the solid substance,

other factors have to be eliminated, such as the time of penetration of salt into the tissues, which is a function of the purity and concentration of the salt and the thickness of the tissues. This can be avoided by using muscle press juice extracted at low temperature from the flesh shortly after the death of the fish.

Preparation of Press Juice

Living codfish were brought ashore, washed and filleted. Fillets from 6 to 8 fish were minced for each lot. The minced flesh was then put in a special press capable of holding about 6 kg. of flesh and yielding about 1,000 ml. of juice in half an hour at 5°C.

After thorough mixing, the juice was distributed into flasks containing c. p. sodium chloride in amounts sufficient to give the desired concentrations. These were shaken to dissolve the salt and stored in constant temperature boxes. Duplicate flasks were put up in all cases, one of which was used for chemical analysis, the other for bacteriological tests.

Determination of Volatile Bases

Total volatile bases and trimethylamine were determined by distillation in vacuum by a method similar to that described by Beatty and Gibbons (1937). With our special apparatus four determinations, two of total bases and two of trimethylamine, could be made at the same time. All distillations were made at room temperature, at a pH of 9.7 using a 2 per cent solution of potassium carbonate to free the bases, and the distillations carried out for 20 minutes. By using N/42 acid and alkali solutions, and a burette graduated with 0.01 ml. subdivisions, the method was found accurate enough to follow small changes in the course of spoilage.

In the determination of trimethylamine the procedure was the same except that formaldehyde was added to the sample to retain other bases. Throughout this paper trimethylamine refers to this fraction which is not retained by formaldehyde and which is believed to be trimethylamine (Beatty and Gibbons 1937).

Bacterial Counts

Ordinary beef extract agar, to which were added 1.5, 10 or 20 g. of sodium chloride for each 100 ml. of broth, was used in making counts. These agars are referred to throughout the paper as 1.5, 10 and 20 per cent agar. One ml. samples were used in all cases. Dilutions were made in brine of the same strength as the agar used in plating, i.e., 1.5, 10 or 20 per cent. All plates were incubated at 25°C. Counts on 1.5 per cent agar were made after six days, on 10 per cent agar after 14 days, and on 20 per cent agar after 21 days. This length of time allowed the maximum number of colonies to develop.

EXAMINATION OF FRESH JUICE

Total Proteins

In four lots analyzed the apparent protein content of the juice as determined by multiplying the total nitrogen by 6.25 was 7.31, 8.20, 7.00 and 7.56, giving an average of 7.5 per cent.

This amount of protein in the juice, about one-third of the protein in fish muscle, gives a liquid which is handled easily and may be pipetted accurately, and at the same time contains enough protein to give a fairly good idea of the changes going on in whole fish under the same conditions.

Total Volatile Bases

The amount of volatile bases in fresh juice varies with different lots. In 10 samples the volatile bases expressed as mg. per 100 ml. of juice were 8.25, 9.50, 10.50, 9.27, 8.00, 8.52, 9.00, 9.25, 8.22 and 8.20, with an average of 8.87 per cent.

These determinations were made on fish caught from September to July and the variations are probably due to the condition of the fish. However, as long as the original value is known, such variations are not important.

Trimethylamine

The amount of trimethylamine in fresh juice, as far as the sensitivity of our method could detect, was between 0.10 and 0.20 mg. per cent. There is probably little or no trimethylamine in the flesh of living fish and it most likely comes from a reduction of trimethylamine oxide after death.

Bacteria

The greatest variation in the fresh juice is in the number of bacteria. There are also differences in the types and in the proportions of the different kinds of bacteria. As the flesh of living fish is practically sterile, the bacterial flora is introduced in the handling, largely from the slime and from the intestinal contents. It may thus be seen that it is impossible to duplicate conditions in different lots and in all cases slight differences have been noted in the shapes of the growth curves, the rate of spoilage, etc. The addition of salt makes little difference in these variations, except in the highest salt concentrations where the action is so great that the original flora matters but little.

CHEMICAL AND BACTERIOLOGICAL CHANGES IN SALTED JUICE

The experiments described in this paper have been repeated on three different lots of juice. These experiments were carried out at 10 and 21°C. One experiment was started at 37°C. but the protein denaturation was so great that accurate sampling was impossible. Salt concentrations studied were 8, 12, 16, 20, 24, 28 and 32 per cent expressed as g. of salt added to 100 ml. of juice. The quantity of sodium chloride in the fresh juice does not vary enough to lead to appreciable errors in the final salt concentration. This variation is negligible and does not exceed errors in manipulation. Total bases and trimethylamine are given as mg. per cent nitrogen. The chemical results are corrected, depending on the specific gravity of each sample, giving in all cases the values for 100 g. of fresh juice. Bacterial counts are given per ml. of salted juice, the error in the counts being greater than the correction factor.

For ease of presentation the chemical and bacteriological changes will be given together.

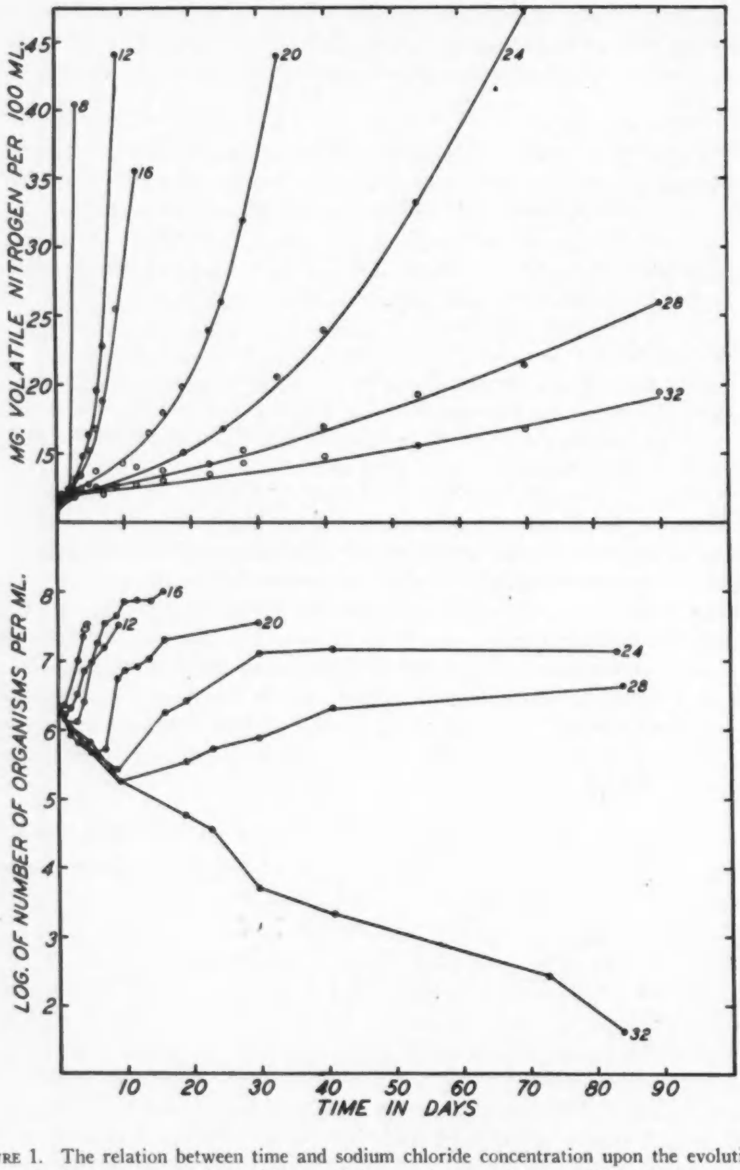

FIGURE 1. The relation between time and sodium chloride concentration upon the evolution of volatile nitrogen and bacterial growth in muscle press juice at 21°C. Sodium chloride expressed as g. per 100 ml. juice.

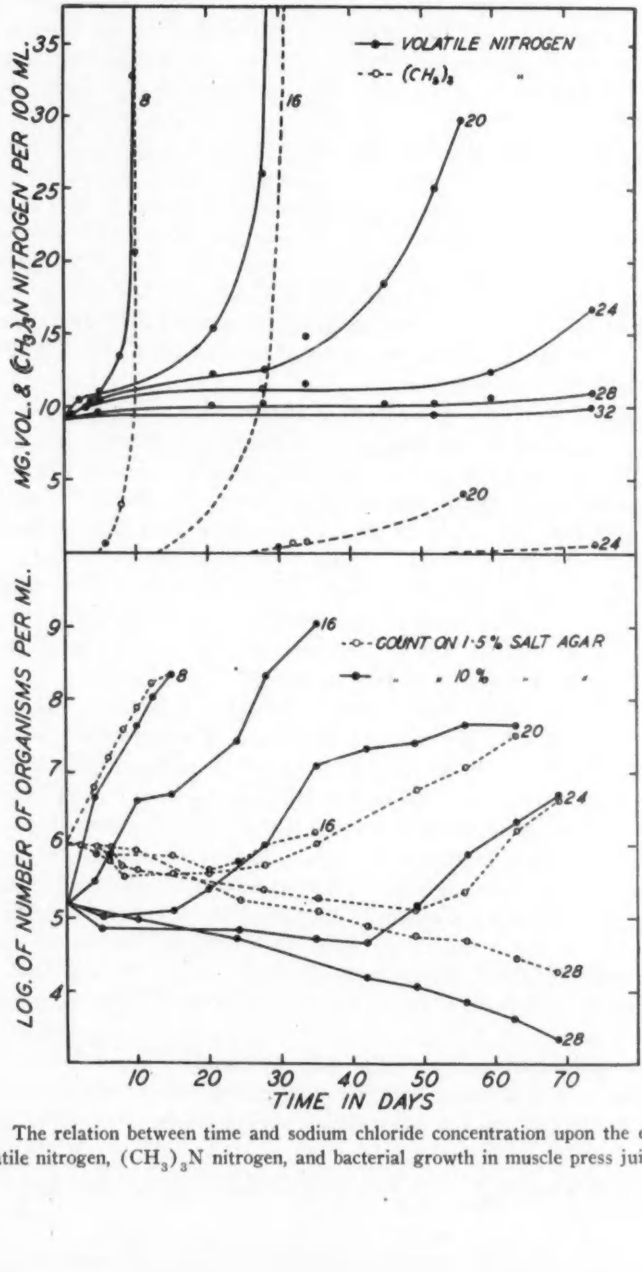

FIGURE 2. The relation between time and sodium chloride concentration upon the evolution of volatile nitrogen, $(CH_3)_3N$ nitrogen, and bacterial growth in muscle press juice at 10°C.

The first series was carried out at 21°C. and the total volatile base production was followed. Figure 1 shows that the rate of total volatile base formation varies inversely with the salt concentration. At the lower concentrations there is a short period of slow production, followed by a rapid increase. As the salt concentration increases the period of slow production lengthens. At the higher concentrations, 28 per cent and especially 32 per cent, the rapid increase never occurs, at least not before 80 days.

In this series bacterial counts were made on agar containing 1.5 per cent salt. This medium, as may be seen on comparing figures 1 and 2, is not very satisfactory, and presents only a partial picture of the bacterial population. There is in all cases a drop in the number of organisms after the salt is added. This drop is very slight and of short duration at the lower concentrations. As the salt concentration increases the decrease in numbers continues for a longer time and at the highest concentration continues beyond the duration of the experiment with apparently no recovery. In no case is there a very great increase in the population, never being more than 100 times the original.

The second series was carried out at 10°C. As may be expected the period of slow production of volatile base is much longer for corresponding salt concentrations. In most cases the lag is from three to four times as long. However, once rapid production begins the rate is as great as at the higher temperatures. At 24 per cent and especially at 28 per cent and 30 per cent this rapid increase has not occurred in 74 days.

Trimethylamine production was also followed in this series. Trimethylamine does not increase for several days at the lower concentrations, but the rate of production is then very rapid. At 20 per cent salt concentration the increase is not as great and at 24 per cent very slight in 74 days.

Bacterial counts were made on media containing 10 per cent and 1.5 per cent salt. As before, the 1.5 per cent count was not very satisfactory except at the lowest concentrations. The decreases in this series were not as great as in the first. These variations may be expected as the original flora in each series was probably quite different both as to species present and their relative proportions.

The original count on 10 per cent agar is lower than the original count on 1.5 per cent agar. There is a slight drop in numbers in the higher concentrations but the lag period is not as great and the subsequent growth is more rapid than on 1.5 per cent agar. It will be seen from figure 2 that the increase in the 10 per cent count corresponds more closely with the increase in volatile base and trimethylamine. The 32 per cent count followed the 28 per cent so closely that it was not included.

In the third series the same lot of juice was used for a run at both 21°C. and 10°C. This insured the same bacterial flora in both cases and furnished a better basis for comparison, although very similar sets of curves were obtained for both volatile base and trimethylamine production as in the first two series. The production was more rapid but this may be because of a greater bacterial population in the original juice.

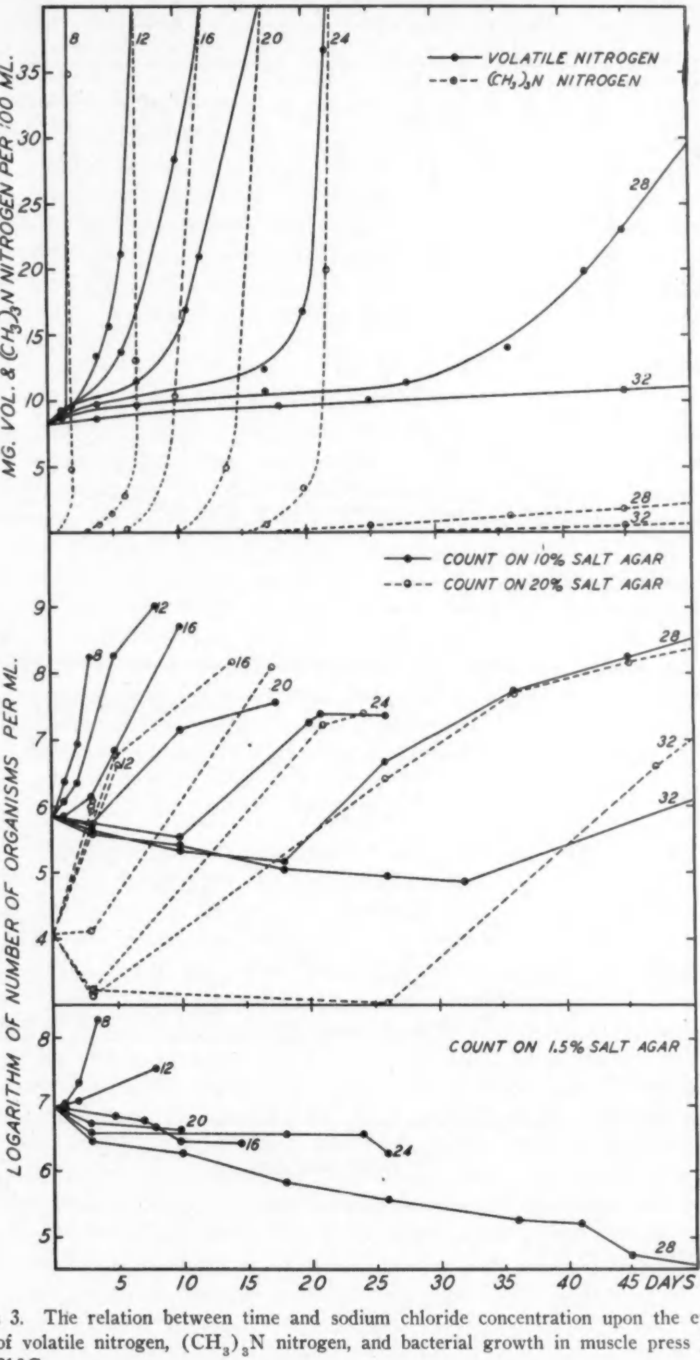

FIGURE 3. The relation between time and sodium chloride concentration upon the evolution of volatile nitrogen, $(CH_3)_3N$ nitrogen, and bacterial growth in muscle press juice at 21°C.

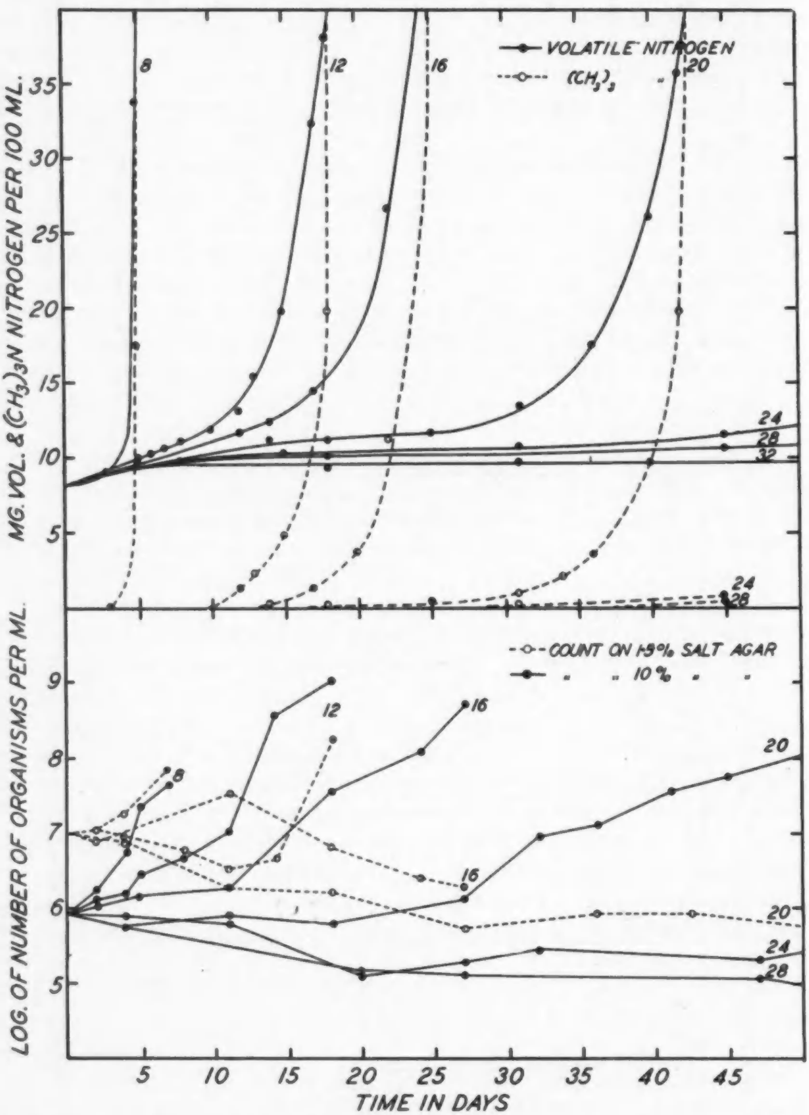

FIGURE 4. The relation between time and sodium chloride concentration upon the evolution of volatile nitrogen, $(CH_3)_3N$ nitrogen, and bacterial growth in the same juice as used in figure 3 but at 10°C.

In this series counts were made on 1.5 per cent, 10 per cent and 20 per cent salt agar. As before the first was of little value except in the 8 per cent salt juice. The original count on 20 per cent agar was much lower than on 10 per cent agar. At 21°C. in juice with 20 per cent salt or more (as shown in figure 3) the lag period as shown by the 20 per cent count was shorter than that shown by the 10 per cent count. The rate of multiplication was somewhat slower so that in most cases the final population as shown on the two agars was about the same.

At 10°C. too few counts on 20 per cent agar were obtained in the first three weeks to plot. Those which were made showed that they followed the same general trend as noted above, with the final count approximating that obtained on 10 per cent agar. The 10 per cent agar count as shown in figure 4 was very similar to that of series two.

Relation of Odour to Volatile Base Nitrogen

In untreated fish muscle juice putrid odours are first apparent when the trimethylamine nitrogen reaches a level of about 4 to 5 mg. per cent (Beatty and Gibbons 1937). In treated muscle juice the odour also makes its appearance when trimethylamine reaches similar levels. That odour is associated with trimethylamine rather than total volatile base is evident because, though the volatile base reached a level of 30 mg. per cent after ten days in a sample containing 28 per cent salt, no odour appeared, yet in samples at lower salt concentration odour appeared at a level of 15 mg. per cent of total volatile base. It will be seen by referring to figure 3 that in the former case the trimethylamine level did not reach 5 mg. per cent, while in the latter it was above that point. It was found that influences which tend to limit the production of trimethylamine, such as low temperature and high salt concentrations, also tend to limit the odour evolved.

DISCUSSION

As there is little or no increase in the number of bacteria growing on 1.5 per cent agar, except at very low salt concentrations, these counts need not be considered. The increase in population as shown by the count on 10 per cent agar corresponds most nearly with the increase in volatile bases and in trimethylamine. On 20 per cent agar, below 20 per cent salt concentration in the juice the increases are not as great as on 10 per cent agar. At 20 per cent and above, the final count on both 10 and 20 per cent agars is practically the same. However, the end of the lag period, as shown by the 10 per cent count, more nearly corresponds to the increase in volatile bases, and our interpretations are based on these counts.

Although not enough work has been done on the types of organisms growing on the different salt agars and various concentrations of brine to warrant definite conclusions, preliminary studies have shown that most of the organisms growing on the higher salt agars prefer either 10 or 20 per cent salt and grow poorly or not at all on 1.5 per cent salt agar. This is particularly true of the rod forms. The coccus forms generally grow better with little salt, but are able to adapt themselves to fairly high concentratoins. All the colonies from a plate or a section of

one were picked in a few instances and grown on the corresponding strength agar. On transferring these to the three different salt agars, it was found that about 50 to 90 per cent were halophilic.

The length of time fish muscle proteins keep depends on the salt concentration and the temperature. At 21°C. a salt concentration of 32 per cent is necessary to prevent spoilage. Twenty-eight per cent salt will keep fish juice only about 35 days. At 10°C., 24 per cent salt will keep juice 60 to 70 days, and 28 and 32 per cent much longer than this. On comparing the bacterial counts it may be seen that in all these cases the number of organisms is either less than the original count or very slightly above it.

The onset of rapid decomposition corresponds with the appearance of trimethylamine. The rise in volatile bases before the appearance of trimethylamine is due almost entirely to ammonia. Once trimethylamine appears the rise in volatile bases may be accounted for by this fraction. In almost every case, if at any time the trimethylamine value is subtracted from the total volatile bases, we get for the ammonia curve a straight line, a linear function with the time, except for advanced decomposition.

Probably the most interesting observation is that the appearance of trimethylamine, and hence the beginning of the rapid increase of volatile bases, coincides with that point when the logarithm of the bacterial count reaches 7 or 7.25, or, in actual numbers, when the count is from 10 to 20 millions per ml.

From the above observations and from observations on fresh fish as reported by Beatty and Gibbons (1937) the following theory is advanced. The production of ammonia is due to enzymatic action. As salt is added the enzyme action is repressed although bacterial growth may still take place (Rockwell and Ebertz 1924). As bacteria develop in fish and similar unaerated media the electrode potential falls, until finally a reducing system is set up which reaches its maximum at about the end of the logarithmic phase of growth (Hewitt 1936). In fish, when the reduction potential reaches a certain point, the trimethylamine oxide present is reduced to trimethylamine. This reduction begins when the bacterial population reaches about 10 or 20 million per ml. While this is not at the end of the logarithmic growth phase in the lower salt concentrations, it is near this point for the higher concentrations if the count on 10 per cent salt agar is considered. The fact that the appearance of trimethylamine is so rapid and that once initiated is almost independent of the salt concentration also points to a reduction mechanism. Stuart and James (1937) suggest that salt adds poise to a medium. This may explain why a greater production of trimethylamine was not obtained at some of the higher salt concentrations even when the bacterial count had reached 10 to 20 million per ml. It is believed that all the trimethylamine oxide is reduced very rapidly. This leads to the very decided odours associated with spoiled fish, although the actual protein breakdown may be very slight. This hypothesis is being investigated at the present time.

SUMMARY

Chemical and bacteriological changes have been followed at 21 and 10°C. in fish muscle press juice containing salt in amounts varying from 8 to 32 per cent. The rise in bacterial population as measured by the count on 10 per cent salt agar corresponds to the rise in volatile bases. Counts on 1.5 per cent salt agar are not satisfactory. Counts on 20 per cent salt agar agree fairly well with 10 per cent counts although the lag period is not as great at the higher salt concentrations, but the rate of growth is such that the final numbers are practically the same.

After the lag period, the rate of production of volatile bases is about the same for all concentrations showing an increase. This rapid increase is due almost entirely to trimethylamine. A hypothesis is advanced that this is due to a reduction of trimethylamine oxide, initiated by a reduction potential set up by the bacteria present when their numbers have reached between 10 and 20 million per ml. as measured by the 10 per cent count.

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The Food and the Food Supply of Sockeye Salmon (*Oncorhynchus nerka* Walbaum) in Cultus lake, British Columbia.*

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ABSTRACT

The analysis of several hundred stomachs of sockeye salmon taken from Cultus lake at all seasons shows that pelagic plankton crustaceans are practically the only food of the fingerlings, and are the most important food of the older fish. The total food consumed by fingerlings, per unit bulk, is greatest in July and August, least in winter. The entomostracan food available in the lake, as determined from plankton samples, varies seasonally in a similar manner. The percentage utilization of each of the four species of Entomostraca by a fingerling sockeye population is related directly to the plankter's abundance, and inversely to its size. Individual sockeye, however, occasionally exhibit preference for smaller or less abundant species.

The summer feeding of fingerlings appears to be chiefly confined to the region between 5 and 15 metres depth, which includes the thermocline and adjacent narrow strips of the epilimnion and hypolimnion. Foraging is limited upward by scarcity of food, and downward by poor illumination or low temperature.

Other fish in the lake are known to compete with sockeye for plankton, but such interspecific competition is believed to be quantitatively of minor importance, in summer at least. Intraspecific competition for food in years of large sockeye populations is sufficient to reduce their rate of growth, and is probably responsible for an unusually early decline, in those years, of the summer's supply of Entomostraca.

INTRODUCTION

This paper presents the results of a study of the food of the young sockeye salmon in Cultus lake, undertaken in an attempt to reveal more clearly the relation of the young fish and their development to the available food supplies in the lake. In addition the general relationship of sockeye to other species of fish in the lake is discussed, particularly in regard to competition for food.

The sockeye salmon inhabiting Cultus lake consist of fish in their first year (fingerlings), in their second year (yearlings), and a number in their third and fourth year of life. The majority migrate seaward from the lake in April and May as year-old fish, but a small fraction varying from year to year are two-year-olds. The number of each sort in the various migrations since 1926 have been tabulated in the publications of Foerster (1929, 1934a, 1934b, 1936a, 1936b).

METHODS OF OBTAINING AND ANALYZING STOMACH CONTENTS

Although the capture of sockeye during migration is not difficult, there is still no altogether satisfactory method of obtaining a representative sample of

*No. 2 of a series: "Factors affecting the behaviour and survival of sockeye salmon (*Oncorhynchus nerka* Walbaum) during their lacustrine existence, in Cultus lake, British Columbia."

them while they are resident in the lake. Two methods have been employed in this investigation. Bottom-set gill nets were successful in catching a few specimens, chiefly yearlings and older fish, the sizes of mesh used being 16, 22, 27 and 38 millimetres stretched ($\frac{5}{8}$, $\frac{7}{8}$, $1\frac{1}{16}$ and $1\frac{1}{2}$ inches). A much greater number of sockeye, largely fingerlings, have been taken from the stomachs of predaceous fish.

The stomachs of sockeye taken by both of these methods were examined in order to obtain as complete as possible a picture of their food at all seasons of the year. The contents of stomachs only, not of the intestines, were used. When any considerable bulk was present, (usually from a number of fish taken at the same time), it was pressed fairly dry between filter papers and weighed, the result being the *wet weight* of the food. The material was then mixed with water, diluted to known volume, and a fraction counted in the same manner as for plankton samples (Ricker unpublished MS.). In order to facilitate comparison of the different organisms, they have been *weighted*, on the basis of their average bulk as follows:

Epischura:	10 units
Cyclops:	1 unit
Daphnia:	5 units, May to September
"	3 units, October to April
Bosmina:	1 unit
Midge pupae:	50 units

Comparison with weighed samples shows that 1 unit is equivalent to about 0.01 mg. wet weight.

RESULTS

The Food of Sockeye in Their First Year

SEASONAL VARIATION IN QUANTITY CONSUMED

In table I are given the kinds and quantities of organisms found in the stomachs of fingerling sockeye. In taking averages, empty stomachs have been included with those containing food. Figure 1A indicates graphically the consumption of plankton by fingerling sockeye in 1932-33. The average wet-weight of stomach contents is small in May, increases quickly throughout the summer to as much as 31 mg., falls off in autumn to between 5 and 10 mg. in January, to increase again to about 35 mg. in April and May.

Consideration should, however, be given to the increasing bulk of the fish over this time. Few individuals were in condition to be weighed, but in many cases length could be determined, as given in the third column of table I. Since the weight of the fish may be considered approximately proportional to the cube of their length, figure 1B, in which the bulk of the stomach contents is divided by the average of $(\text{length})^3$, has been constructed to show that the time of most active feeding is in July and August, identical with the season of most rapid growth. A numerical comparison by the "unit" system above gives practically the same picture.

Temperature and food available are two obvious factors which could affect consumption of food by the sockeye, length of day being possibly another.

The writer's observations on the first two (Ricker 1937 and unpublished MS.) indicate that the period May to August would be the optimum time for a large

TABLE I. Stomach contents of sockeye in their first year of life. Horizontal dotted lines separate the different year-classes. Specimens were obtained from stomachs of predatory fishes, except as noted

	Number of stomachs examined	Average length of sockeye	Average weight of contents	ENTOMOSTRACA					Average number midges per stomach	Average food "units" per stomach	Other items
				Total number per stomach	*Epischura*	*Cyclops*	*Daphnia*	*Bosmina*			
		mm.	mg.		%	%	%	%			
1932											
Jan. 9	6	-	-	870	0.9	6	11	77	-	1140	5% Ostracoda
Feb. 4-5	9	-	-	700	1.1	41	8	50	-	900	
" 26	5	-	-	1500	0.5	66	10	24	-	1870	
Mar. 22	19	-	-	1160	1.5	21	56	22	0.01	2620	
Apr. 29	11	-	-	1820	0.0	30	18	52	0.09	2480	1 Aphid
Apr. 20 to May 5[x]	90	90	10.7	420	0.1	27	5	68	0.13	580	
....											
May 10	15	-	-	10	-	3	5	92	-	12	
" 18	50	23	0.3	15	0.4	10	30	59	0.04	26	
June 3	9	-	0.7	24	0.8	2	88	10	-	66	
July 14	58	36	4.5	210	0.6	5	92	2	-	990	
" 31	52	44	14.3	330	2.0	15	82	1	-	1490	
Aug. 25	40	54	31.1	540	0.4	11	89	-	-	2460	
" 25[xx]	11	69	-	1000	1.4	4	93	2	-	4840	
Sept. 9	6	-	-	80	1.1	13	84	1	-	380	
Oct. 10	14	-	10.7	730	0.7	72	16	11	0.07	1010	
Nov. 18 to 20	56	63	21.6	1930	1.8	52	3	43	0.04	2360	
1933											
Jan. 13	9	-	6.1	-	-	90	-	10	-		
" 28	28	60	9.6	450	5.2	85	2	8	0.03	670	
Feb. 17	1	-	-	540	0.7	84	0	15	1.	630	
Mar. 1	1	-	-	780	0.4	83	1	15	-	820	
" 17	10	73	13.0	1150	1.0	91	1	7	0.8	1330	
Apr. 12	26	67	36	1940	0.1	92	2	6	0.2	2040	
May 2	9	62	22	2040	0.0	97	1	2	-	2060	
" 20	4	-	45	2750	0.0	79	3	18	-	2890	
....											
1934											
July 4	3	47	8	90	4	-	95	1	-	480	
" 23	4	55	-	400	2	9	87	2	-	1920	
Aug. 29	2	70	-	60	5	4	91	-	-	280	
Sept. 19	5	69	38	900	3	16	80	1	-	2570	
Oct. 2	1	78	-	650	6	19	55	20	-	1760	1 Cicadellid 1 Hymenopter
" 10	1	-	-	2670	1	63	5	21	-	2910	

[x] Consist of migrating salmon from the outlet
[xx] These fish were taken in a gill net

consumption. The observed stomach contents agree in general with this contention, except that the small sockeye of May and early June which had just commenced their free-swimming life in the lake, ate less per unit bulk than would be suggested by the abundant food available.

The decrease in food consumed toward the close of summer coincides with a pronounced decrease in rate of growth, and the relationship is very probably a causal one. Below the epilimnial layer, where the sockeye are resident, the temperature is increasing up to late October at least. Their food however, as measured either by stomach contents or total quantity available in the lake, drops off sharply with the final disappearace of the summer's pulse of *Daphnia* in September.

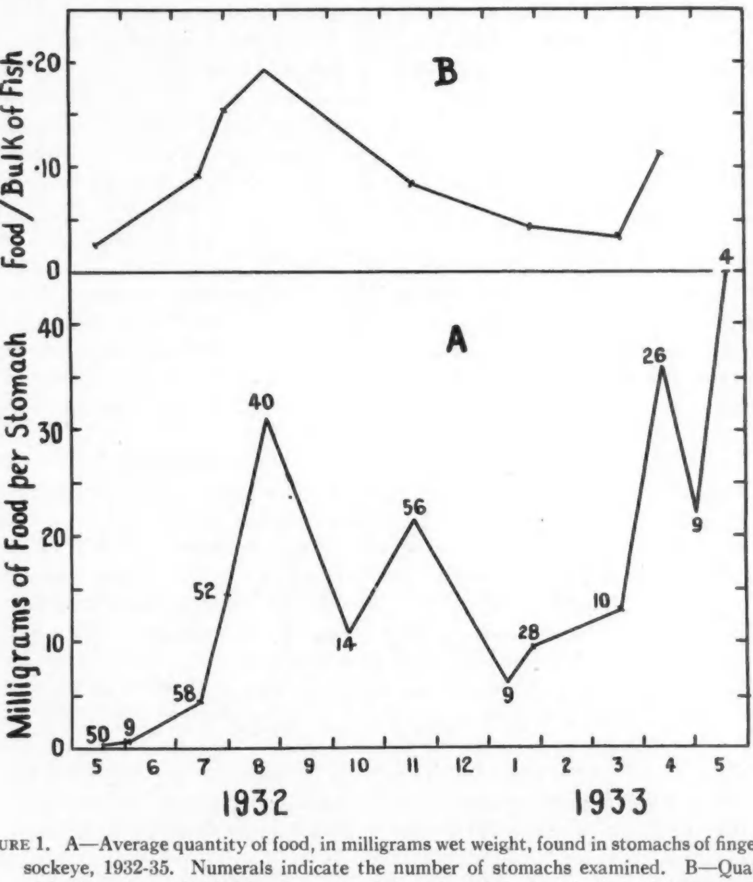

FIGURE 1. A—Average quantity of food, in milligrams wet weight, found in stomachs of fingerling sockeye, 1932-35. Numerals indicate the number of stomachs examined. B—Quantity of food as above, divided by the mean of the cubes of the length of the fish, in mg./cm.[3].

SEASONAL VARIATION IN KIND CONSUMED

The foods found in sockeye fingerlings consist predominantly of the four pelagic plankton crustaceans: *Epischura nevadensis*, *Cyclops bicuspidatus*, *Daphnia pulex* and *Bosmina obtusirostris*. Midge larvae, almost entirely Chironominae, are of some importance. Other items found in insignificant quantities are the

diatom *Melosira*, Ostracoda, and small terrestrial insects (Aphididae, Cicadellidae, Hymenoptera), while *Notholca* was found several times by Foerster (1925).

The seasonal occurrence of the four entomostracans may be seen in table I. *Epischura* is never a common organism in the stomachs, representing usually about one per cent of the total number of entomostracans; its large size makes it of relatively greater importance than its small numbers suggest. *Cyclops* is taken at all seasons, comprising up to 90 per cent of the organisms in winter and early spring, but in summer it may be only about 5 per cent. *Daphnia* is the commonest food of summer and early fall, and was fairly common in the winter of 1932. *Bosmina* is taken most commonly by the small fingerlings in May, and appears again in good numbers in late autumn and to a lesser extent in winter.

The relative utilization of the four organisms by the sockeye is better shown in figure 2, where the relative numbers of the plankters are plotted. The abundance of each species in the stomachs (as a percentage of the total number of Entomostraca) is compared with their abundance in the lake at the same time, the latter values having been obtained from total vertical net hauls. Even making every allowance for the small size of some of the samples, it is evident that *Daphnia* is the organism most readily taken by the sockeye, except when the latter are very small (May and June). Next in order of utilization is *Bosmina*, which is also the form most often occurring in the youngest fish. The small *Cyclops*, although consumed in large quantities when Cladocera are scarce, does not command sufficient attention when they are commoner to be eaten in significant numbers. *Epischura* is much more intensively utilized in winter than in summer, compared to its abundance in the two seasons.

From this evidence, four factors can be postulated to account for the extent of predation of sockeye upon different plankters. *Abundance* of the plankter is abundance beyond which it ceases to be of significant value. *Size* is important, as both with Cladocera and Copepoda the larger organism is taken more frequently than the smaller in relation to its abundance. *Habitat selection* appears the most probable explanation of the fact that *Epischura*, although as large as naturally of prime importance. There exists for each species a lower limit of *Daphnia*, is much less intensively used in summer: at this season it inhabits the epilimnion principally, where the other crustaceans are relatively scarce (figure 3), and there is consequently little to attract foraging sockeye. A fourth factor must be postulated to account for the fact that *Bosmina* is more intensively exploited than *Cyclops*, although the two are of much the same size and occupy similar habitats. This might be because of a greater agility or lesser visibility on the part of *Cyclops*, different diurnal migrations, or simply a preference for *Bosmina* exhibited by the majority of the sockeye. The last possibility is discussed again below.

Food of Sockeye in Their Second Year

A small number of yearling sockeye were taken from the lake, and an analysis of their stomach contents is presented in table II. As with fingerlings, entomostracans are the principal food, with *Daphnia* much more common than all other

species, in summer. In spring and fall the smaller entomostracans are more important, and at these seasons the fish also take large numbers of higher arthropods: Chironomid pupae, Aphididae, various small Diptera, Homoptera, Coleoptera, Hymenoptera and Acarina. Because of their much larger size, the yearlings

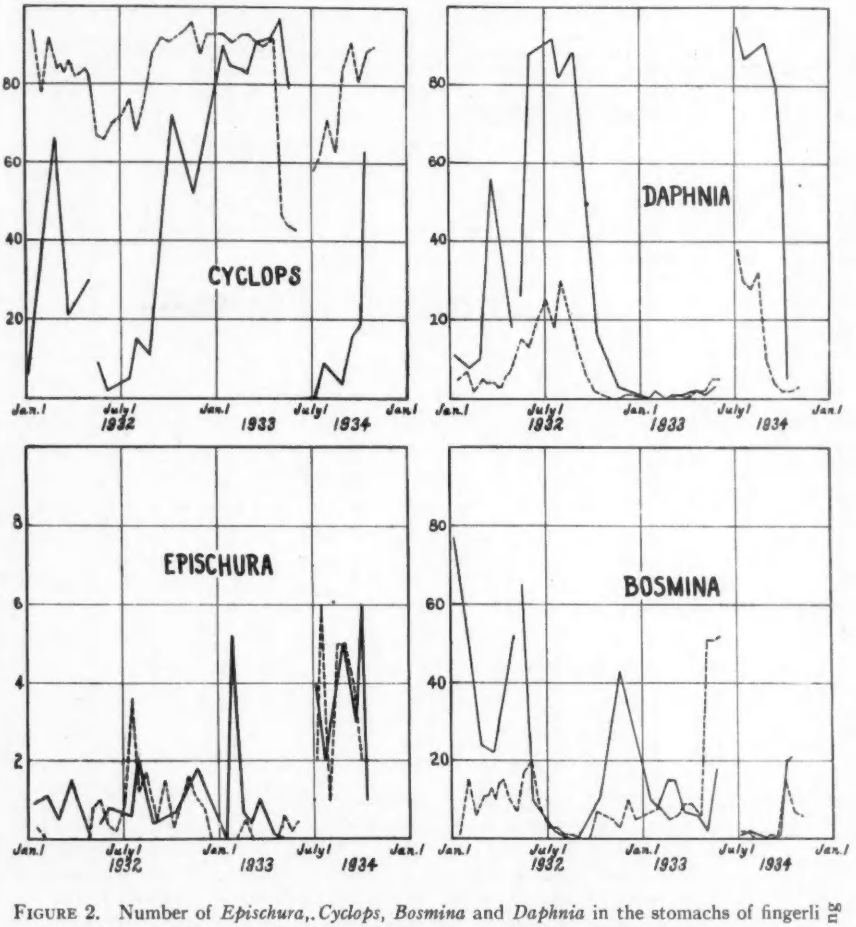

FIGURE 2. Number of *Epischura*, *Cyclops*, *Bosmina* and *Daphnia* in the stomachs of fingerling sockeye (solid line), and in the pelagic region of the lake (broken line), expressed as a percentage of the total number of adult entomostracans present, in each case. Note that the ordinate scale for *Epischura* is ten times that of the others.

contain considerably more food per individual than do the fingerlings, although empty stomachs are more frequently found than in the latter. The data are however not sufficient for a numerical comparison.

The net-caught fish of November 18, 1932, were mature "residual" sockeye, which had matured in the lake in their second year of life. Their stomachs were

mostly empty, but a few contained imaginal midges and terrestrial insects. Another group of maturing second-year residuals was taken in 1934, but earlier in the season—June 14 to September 26. The nine examples examined had eaten the two large species of entomostracans, with a greater average percentage of *Epischura* than was observed among fingerlings.

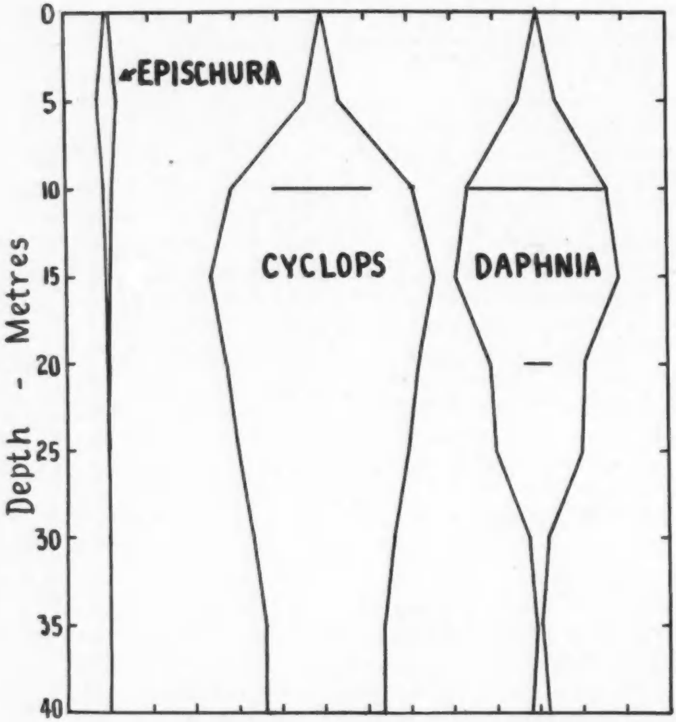

FIGURE 3. Vertical distribution of Entomostraca, by day, in the pelagic region of Cultus lake, during July, 1934. Values indicated are the mean of three series of nine trap-catches each, taken at the beginning, middle and end of the month. Each division of the abscissal scale represents 2 organisms per litre; note that it is not of the "spherical" type often used in plankton diagrams. Horizontal lines at 10 and 20 metres depth represent the relative number of each species eaten by sockeye which were held for two days at those depths, July 16-18, 1935.

The last-mentioned observation suggests that the yearlings make more frequent excursions into the epilimnion than do the fingerlings, for it has been found that *Epischura* is the only one of the large plankters of the lake which has its greatest population density in this layer of water. Their tendency, in 1932, to take insects from the surface of the water, is even more convincing evidence of the same.

TABLE II. Stomach contents of sockeye in their second-year of life. Figures in brackets are estimated. All specimens examined in 1934, had testes enlarged, and would have matured the same autumn.

	How taken	Number of stomachs examined		Av. length of sockeye	Av. wet weight of contents	ENTOMOSTRACA					OTHER ITEMS		
						Total number per stomach	Epischura	Cyclops	Daphnia	Bosmina	Kind	Av. no. per stomach	% of total weight
1932		Full	Empty	mm.	mg.		%	%	%	%			
July 29	Net	1	0	125	-	50	-	-	100	-	Midge pupae	1	?
Aug. 25	"	1	0	115	-	150	-	-	100	-	-	-	-
Oct. 12	"	3	2	119	100	6	+	-	30	70	Land insects#	50	100
" "	"	1	0	172	100	-	-	-	-	-	Midge pupae	4	20
											Aphididae	5	20
											Bibionidae	6	60
Oct. 12	Stom.	1	0	(130)	-	570	2	9	2	87	-	-	-
Nov. 21	"	1	0	104	10	200	-	-	-	100	-	-	-
" 18x	Net	5	13	191	20	-	-	-	-	-	Midge imagos	5	95
											Land insectsxx	+	5
1933													
Mar. 1	Stom.	1	0	122	230	5600	11	76	10	3	Midge pupae	63	95
											Aphididae	5	2
											Spiders	2	3
" 17	"	1	0	(125)	60	-	-	-	-	-	Cercopidae	2	30
											Coleoptera	1	10
											Spiders	1	60
Oct. 12	Net	1	1	119	60	1300	2	-	98	-	-	-	-
1934													
June 14	Net	1	0	96	110	1240	2	-	96	2	-	-	-
July 4	"	2	0	96	50	300	31	-	69	-	-	-	-
" "	Stom.	1	0	(85)	-	(100)	-	-	100	-	-	-	-
" 19	"	2	0	102	-	90	-	-	100	-	-	-	-
Aug. 29	"	2	0	(130)	230	1400	18	-	82	-	-	-	-
Sept. 26	"	1	0	104	50	680	2	-	96	2	-	-	-

\# Mostly Aphididae (98% wt.), but also other Homoptera, Heteroptera and Diptera
xx Includes Mycetophilidae and Hymenoptera
x Mature male "residual" sockeye

FOOD OF SOCKEYE IN THEIR THIRD YEAR

In table III is presented the stomach analysis of a group of sockeye in there third year of life, taken in 1934, and all maturing residual males. These were separated from the similar second-year fish of the same year by scale-reading when possible, and by length in other cases. Evidence from the lengths observed was that there would be little overlapping in size between the two groups. Like the second-year fish, the bulk of their food is of entomostracans, among which only *Daphnia* and *Epischura* are important. A new item consists of a large number of small fish, taken in two stomachs, and identified in one case as larval *Cottus*.

TABLE III. Stomach contents of sockeye in their third year of life. All specimens had enlarged testes, and would have matured the same autumn

								ENTOMOSTRACA				OTHER ITEMS		
		How taken	Number of stomachs examined		Average length of sockeye	Average wet weight of contents	Total number per stomach	*Epischura*	*Cyclops*	*Daphnia*	*Bosmina*	Kind	Average number per stomach	% of total weight
			Full	Empty	mm.	mg.		%	%	%	%			
1934														
June	14	Stom.	1	0	160	110	300	50	–	30	20	Small fish	15	90
"	"	"	1	0	122	100	1200	–	–	100	–	–	–	–
"	26	Net	1	2	162	80	650	8	1	91	–	–	–	–
July	4	"	1	0	138	350	150	?	–	100?	–	Midge pupae	1	+
												Larval *Cottus*	51	99
"	10	"	2	1	171	190	2600	2	–	98	–	–	–	–
"	23	"	0	1	172	0	–	–	–	–	–	–	–	–
"	31	"	2	0	177	300	2600	1	5	94	–	–	–	–
Aug.	7	"	0	2	186	0	–	–	–	–	–	–	–	–
"	7	Stom.	1	0	170	380	4500	–	3	97	–	–	–	–
"	29	Net	1	0	220	640	3600	3	–	97	–	–	–	–
Sept.	19	"	2	2	216	40	470	4	–	96	–	–	–	–
"	26	"	1	2	219	100	800	–	–	98	2	–	–	–
Oct.	10	"	1	0	246	180	2900	11	–	86	3	Midge pupae	7	30
												Land Diptera	1	2

RATE OF DIGESTION OF ENTOMOSTRACA

In 1935 an attempt was made to ascertain the rate of digestion of plankton crustaceans by sockeye, at temperatures between 11.5° and 13.5°C. For this purpose a group of 50 hatchery-reared fingerlings was selected, of lengths ranging from 38 to 48 millimetres (mean 43.0, standard deviation in length ±2.4). On July 16 they were placed in a screen cage made of two hatchery baskets nailed together, and suspended in the pelagic region of the lake at a depth of 10 metres and temperature of 15.0°C. After 48 hours they were removed, and placed in a hatchery trough fed by plankton-free creek water, the actual transfer occupying 15 minutes in water at 19.5°C. A sample of 10 fish was taken immediately after transfer, and again after the passing of 4 and 8 hours. The stomach contents of each fish were examined separately, and the organisms present enumerated in toto, as in the following schedule:

	Sample 1	Sample 2	Sample 3
Time taken	4.15 p.m.	8.00 p.m.	12.15 a.m.
Temperature of water	13.4°C.	12.0°C.	11.6°C.
Number of empty stomachs	0	5	9
Mean number of *Cyclops*	45	15	1
Mean number of *Daphnia*	65	5	1
Average total food, units	370	36	5
Standard deviation	±150	±55	±14

The progress of gastric digestion is indicated by the appearance of empty stomachs after 4 hours, and their preponderance after eight. At the latter time

the average number of units of food (*Daphnia* being rated as 5 units, *Cyclops* as 1) has declined to about 1 per cent of the original amount—a value so low as to indicate, in spite of its rather high sampling error, that gastric digestion is practically complete.

It should be noticed, however, that the number of food units contained in these sockeye at the beginning of the fast was only about one-fifth of the average quantity contained in wild sockeye of similar size, in 1932 (table I). Hence, to digest the average stomach content of the wild fish at these temperatures would probably take somewhat longer. Wild sockeye may also spend most of their time in water colder than 12°C. A conservative estimate of the food consumption of wild sockeye fingerlings in summer can be had by postulating that their average observed stomach contents pass through the pylorus in about a day's time.

Natural Feeding Areas

The question of what level in the lake the sockeye frequent when feeding is one on which there is still no direct evidence, but suggestive data were obtained at the time of the digestion experiment outlined above. In addition to the cage at 10 metres depth, there was placed in the lake on July 16 a second cage containing 50 sockeye of the same stock, at 20 metres depth. Ten fish of this group were killed two days later, at the same time as the "Sample 1" sockeye of the schedule above. The food of both series is shown below:

	10 metres	20 metres
Temperature	15.0°C.	6.8°C.
Number of empty stomachs	0	5
Mean number of *Cyclops*	45	0
Mean number of *Daphnia*	65	12
Average total food, units	370	60
Standard deviation	±150	±90

The difference in the average food consumed at the two depths, when tested by Fisher's "t" distribution, is very definitely significant. Evidently feeding was much less active at the deeper and colder level. The lower temperature was perhaps partly responsible for this, but the qualitative difference—complete absence of *Cyclops* at 20 metres—suggests that decreased illumination played a part. *Cyclops* are ordinarily as abundant at 20 metres as at 10 metres, in summer, as is evident from figure 3. But since they are much smaller than *Daphnia*, it is probable that illumination at 20 metres did not make them sufficiently visible to the sockeye to be captured.

It seems likely, therefore, that most of the summer feeding of wild sockeye fingerlings is done at levels higher than 20 metres, and probably between 15 metres and about 5 metres, which includes the whole of the thermocline, a narrow strip of hypolimnion, and part of the epilimnion. Extensive feeding in the upper epilimnion seems barred by the scarcity of food there (figure 3), unless diurnal migrations of the crustacea are sufficient to add greatly to the number inhabiting the region in early morning or late evening. Experimental retention of artifically-fed fingerlings in the epilimnion for long periods has resulted in their death, pre-

sumably from the high temperature, but this would not necessarily prevent the wild fingerlings making foraging expeditions as far up into the warm water as the food available would warrant.

Selection of Food

The idea that fish which consume animal plankton obtain their food by passive filtration of the water, now finds little support, having been disproved in several cases. For example, Battle et al. (1936) report that the feeding of herring is by an "act of capture" of each of the individual zooplankters. This type of feeding has also been frequently observed in captive sockeye.

The related question, of whether or not a fish can distinguish between and select one of two or more different kinds of plankters in the same environment, is still controversial. If an environment contains two species of plankters, both potential food organisms to sockeye, the proportion of each occurring in the stomachs of all sockeye feeding there should be the same (within the limits of statistical sampling error) provided feeding is purely random and non-selective. The numerical ratio of the two plankters in the stomachs need not be the same as in the environment, but rather it will correspond to their relative *availability*, which is a compound of abundance, conspicuousness and activity. Conversely, if two sockeye feeding in the same environment are found to contain significantly different proportions of two plankton foods, it must be concluded that they are able to distinguish the two kinds, and that at least one of the two sockeye has actually selected one of the plankters on a basis other than its availability. Such a sockeye can fairly be said to have a preference for that plankter.

To demonstrate the actual occurrence of food preferences among sockeye, the contents of the individual stomachs of the 10 experimental fish held at 10 metres depth to July 18, 1935, are indicated below:

Stomach number........	1	2	3	4	5	6	7	8	9	10
Number of *Daphnia*.....	132	26	61	97	33	8	70	72	80	69
Number of *Cyclops*......	3	0	31	1	1	394	0	5	13	1

In the case of numbers 1, 2, 4, 5, 7, 8 and 10, few or no *Cyclops* were taken. With number 9, and better, number 3, *Cyclops* were taken in significant quantity, though still in a minority. In the case of number 6, *Cyclops* is the principal species, with *Daphnia* a very poor second. Since all of these fish, plus 40 others, were confined in the space of a fifth of a cubic metre, all would seem to have had access to the same absolute and relative numbers of the different food organisms. The great preponderance, in most cases, of *Daphnia* over the equally abundant *Cyclops*, might possibly be explained on the basis of relative availability: the larger animal is doubtless more conspicuous, and might be less active and more easily caught, than the smaller. But for one sockeye to depart from such procedure so strikingly as did number 6, in selecting the smaller organism, calls for quite a different explanation. It is difficult to escape the conclusion that all the sockeye were well aware that two kinds of food were present, and that individual idiosyncrasy led one to prefer *Daphnia*, another to prefer *Cyclops*.

A similar state of affairs occurred among the ten fish killed, in the same

experiment, after four hours of fasting. Of the five stomachs which still had food, four contained from 3 to 22 *Daphnia* and no *Cyclops*, while the other had 146 *Cyclops* and 3 *Daphnia*.

That yearling and older sockeye also may select their food is suggested by the examination of their individual stomach contents, but is not readily apparent from tables II and III, which treat of *average* values as a rule. Even when great differences in the composition of stomach contents of fish caught the same day are evident, it can always be argued that the different fish were in different parts of the lake, where the relative availabilities of the different organisms corresponded to their relative numbers as observed in the stomachs. Repeated instances of food differences, however, lead one to question the adequacy of this explanation. For example, a proportion of *Epischura* as high as that shown in the first stomach of table III has never been approached in plankton samples, and it was observed that all of these were in the fore part of the stomach, unmixed with the other plankters.

The existence of preferences for different food organisms by different sockeye is of interest in itself, and is a warning as to the reliability of the averages of food consumed, when the number of stomachs examined is small.

Plankton Available in Different Years

In an other report (Ricker, unpublished MS.) will be presented graphs of the abundance of the important net plankters of Cultus lake, over a number of years, together with a discussion of how the data were obtained and their limitations as a representation of the population of the pelagic region of the lake. The data as regards Entomostraca are given here (table IV), for several years, the figure tabulated being the mean monthly abundance of the organism in question in a top-to-bottom section of the lake at a point where it is 40 metres deep. Months have been arranged into yearly periods extending from May to April, in order to correspond to the time which each year-class of fingerling sockeye spends in the lake.

In addition to the numbers of the various individual organisms, there is tabulated an index of total food available, on a basis similar to that used in stomach analysis (p. 451). The unit ratings for each organism are the same as formerly, except that *Epischura* is 5 rather than 10 units, because the present counts include a relatively greater number of small individuals of this species than were found in the stomachs. In addition, the average size of all organisms is greater in the stomachs than in the general population, so that the "unit" used here would be perhaps half the bulk of the previous one. The fluctuation of the units of sockeye food available is plotted in figure 4.

From table IV and figure 4 it will be noted that the food available to sockeye is greatest from May to July, least from December to February, the former values being roughly six times the latter. The discrepancy may indeed be even greater, as evidence was obtained that *Daphnia*, the important food organism of summer, is able to avoid a plankton net to some extent, and hence its real abundance could be considerably greater than that shown. Although weaknesses of the sampling technique do not allow too definite a conclusion, it is probable that through the

TABLE IV. Monthly average abundance of adult Entomostraca in Cultus lake, expressed as number of individuals per litre

		Epischura	Cyclops	Daphnia	Bosmina	Total	Units
1928	May	1.7	35.0	15.0	2.3	54.0	120.8
	June	1.2	39.0	10.0	3.1	53.3	98.1
	July	1.3	22.0	4.6	2.3	30.2	53.8
	August	0.8	12.0	1.2	1.4	15.4	23.4
	September	0.2	24.0	1.2	1.0	26.4	32.0
	October	0.1	51.5	0.4	1.5	53.5	54.7
	November	0.0	60.0	0.2	1.6	61.8	62.2
	December	0.0	45.0	0.1	1.4	46.5	46.7
1929	January	0.0	30.0	0.0	1.2	31.2	31.2
	February	0.0	17.5	0.0	0.8	18.3	18.3
	March	0.0	13.0	0.0	0.5	13.5	13.5
	April	0.0	10.5	0.1	0.7	11.3	11.5
	Total	5.3	359.5	32.8	17.8	415.4	566.2
	Average	0.4	30.0	2.7	1.5	34.6	47.2
1932	May	0.40	35.0	6.5	7.8	49.7	77.3
	June	0.15	29.5	8.0	5.1	42.8	75.6
	July	0.34	12.0	3.8	0.4	16.5	32.9
	August	0.18	10.5	2.7	0.1	13.5	25.1
	September	0.24	16.5	1.4	0.2	18.3	24.7
	October	0.12	12.8	0.1	0.8	13.8	14.5
	November	0.08	10.0	0.0	0.6	10.7	11.0
	December	0.03	13.0	0.1	1.0	14.1	14.5
1933	January	0.01	11.0	0.0	1.0	12.0	12.1
	February	0.07	16.0	0.2	1.8	18.1	18.8
	March	0.00	15.0	0.1	1.5	16.6	16.8
	April	0.03	15.5	0.3	4.2	20.0	20.2
	Total	1.65	196.8	23.2	24.5	246.1	343.5
	Average	0.14	16.4	1.9	2.0	20.5	28.6
	May	0.16	16.5	1.4	18.6	36.7	43.1
	June	0.18	12.0	3.8	13.8	29.8	45.8
	July	0.42	11.5	5.3	4.6	21.8	44.6
	August	0.57	6.0	5.0	0.5	12.1	34.5
	September	0.50	6.0	3.0	0.1	9.6	23.6
	October	0.15	7.0	2.5	0.2	9.9	15.5
	November	0.10	9.0	1.0	0.4	10.5	12.9
	December	0.08	7.5	0.3	0.4	8.3	9.2
1934	January	0.00	6.5	0.1	0.4	7.0	7.2
	February	0.00	5.8	0.1	0.5	6.4	6.6
	March	0.00	5.8	0.1	0.6	6.5	6.7
	April	0.28	11.5	1.5	3.4	16.7	20.8
	Total	2.44	105.1	24.1	43.4	175.3	270.5
	Average	0.20	8.8	2.0	3.6	14.6	22.5
	May	0.22	13.0	3.3	4.8	21.3	35.3
	June	0.42	7.5	4.3	1.2	13.4	32.2
	July	0.54	7.0	4.1	0.1	11.7	30.1
	August	0.63	7.5	3.0	0.1	11.2	25.6
	September	0.30	9.5	0.5	0.1	10.4	13.6
	October	0.38	14.0	0.6	1.4	16.4	19.2
	November	0.13	10.0	0.5	0.7	11.3	12.7
	December	0.15	6.5	0.3	1.0	7.9	8.9
1935	January	0.12	6.5	0.7	0.7	8.0	9.1
	February	0.02	5.0	0.3	0.6	5.9	6.5
	March	0.02	5.0	0.7	1.2	6.9	8.3
	April	0.01	7.0	0.8	1.8	9.6	11.4
	Total	2.94	98.5	19.1	13.7	134.0	212.9
	Average	0.25	8.2	1.6	1.1	11.1	17.7

three seasons included from 1932 to 1935, there has been a definite decrease in the average quantity of food available. The figures for 1928-29, though of very doubtful reliability, suggest that in that year food was considerably more abundant than in any of the more recent ones. However, variations from year to year in the supply of plankton food are not nearly as great as the observed variations in the numbers of the sockeye which prey upon it.

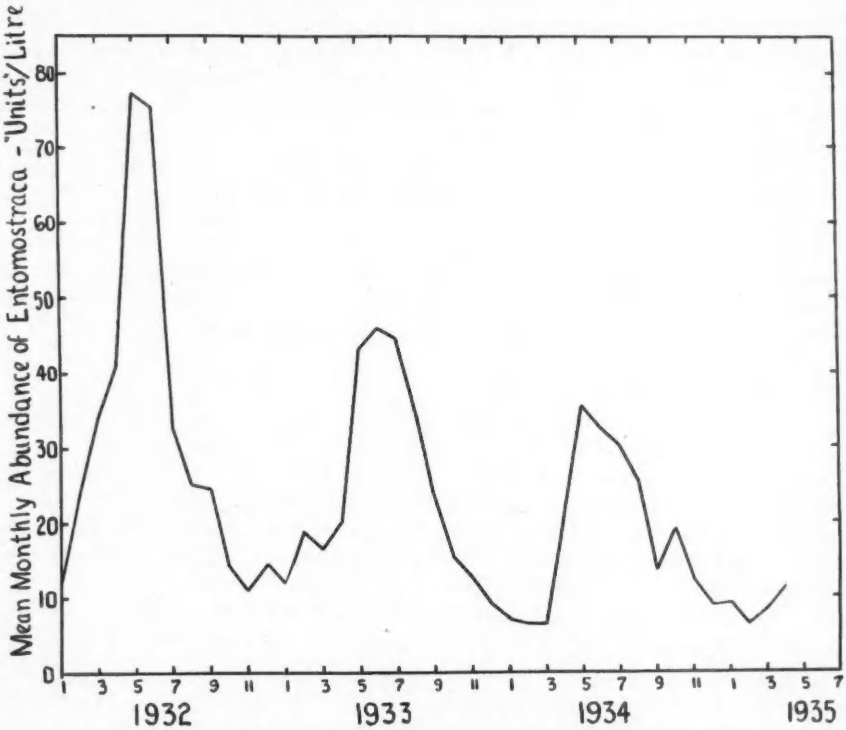

FIGURE 4. Mean monthly abundance of adult Entomostraca in Cultus lake, in "units" per litre. Each species is weighted approximately according to its bulk: *Epischura* = 5 units, *Cyclops* = 1, *Bosmina* = 1, *Daphnia* = 5 (May to Sept.), or 3 (Oct. to April).

Competition of Other Species for Plankton Food

Some mention must be made of the fish other than sockeye which consume plankton in Cultus lake, although the detailed account of their feeding habits is reserved for a separate paper.

Coho salmon (*Oncorhynchus kisutch*) of three age-classes inhabit Cultus lake. The fingerlings are taken readily near shore, and hence probably do not compete with the (pelagic) sockeye during their first summer. Yearling coho make plankton an important food. Third-year coho also consume some plankton, although larger foods are more usual. Cutthroat trout (*Salmo clarkii*) of medium size

occasionally have stomachs full of plankton, in summer. Squawfish (*Ptychocheilus oregonensis*) of medium size consume many *Daphnia* in summer. Both adult and young squawfish appear to frequent the littoral region of the lake at this time. Shiners (*Richardsonius balteatus*) and sticklebacks (*Gasterosteus aculeatus*) are likewise inshore fish in summer, though they may possibly take pelagic plankton in winter. Aleutian sculpins (*Cottus aleuticus*) have been taken only in char stomachs from deep water, and in summer consume considerable plankton, apparently from the bottom water layers. Prickly sculpins (*Cottus asper*) are abundant in shallow water only, and turn to a non-planktonic diet while still quite small. Suckers (*Catostomus macrocheilus*) take large quantities of plankton, especially in spring, but this is probably mostly of littoral origin, as non-pelagic species are present along with the ubiquitous *Daphnia*.

The lake, therefore, contains a number of fish which eat plankton, but during the summer season of rapid growth none of these appear to be serious competitors of the sockeye. The aleutian sculpins may intrude on the sockeyes' feeding grounds from below, and the minnows and sticklebacks impinge on its lateral borders. But throughout the whole pelagic region, comprising four-fifths of the lake's area, the sockeye appear to be without serious rivals. During this time, competition for food, if its exists, is among the sockeye themselves.

Intraspecific Competition for Food

That there may be in some years significant competition among the sockeye themselves for the available food, is evident from several considerations.

The area of Cultus lake has been estimated (Ricker 1937) as 6.26 millions of square metres; its volume as 201 millions of cubic metres. The pelagic feeding grounds available to the sockeye in summer would be more restricted: considered as the stratum lying between the 5- and 15-metre contours, its area is 5.9 millions of square metres, its volume 57 millions of cubic metres.

The number of sockeye fingerlings in the lake at any given time cannot be accurately known, but may be estimated from the data of Foerster. For example, in early summer of 1932 there were about 36 million fingerlings. Such a population fills the available feeding grounds to a density of 6 individuals per square metre of its surface, or two in every three cubic metres of its volume. At the middle of August, this population had probably declined to 16 million, which is two in every 7 cubic metres.*

The principal food of sockeye at that time is *Daphnia*. They were estimated (table IV) to be present in average abundance of 2.7 per litre in August. From this the total number (top to bottom) in the area bounded by the 5-metre contour, is 60×10^{10}, or 37,000 for each of the 16 million sockeye. Of these, about half are smaller specimens than the sockeye usually take, and a large part may dwell in strata where there is insufficient light to make them potential prey. From table I, it can be said that the average consumption of large *Daphnia* by sockeye on

*The original population consisted of 6,000,000 hatchery fry planted in the lake, plus those resulting from the natural spawning of females which contained 50,600,000 eggs of which about $\frac{3}{5}$ are estimated to produce fry. The experimental data used to calculate rate of decrease throughout the summer are contained in a forthcoming paper of Dr. Foerster's.

August 25, 1932, was of the order of 500 per day, and it may have been considerably more than that. It would not be surprising, therefore, to find that in August of this year there was serious competition for food among the sockeye. Similar calculations at other seasons give comparable results; competition may in fact become even more acute during autumn.

Evidence that the consumption of Entomostraca by large sockeye populations may be sufficient to reduce the available supply in late summer may be had from table IV. It will be observed that the August abundance of Entomostraca (on the "unit" rating) is relatively much less in 1928 and 1932, than in 1933 and

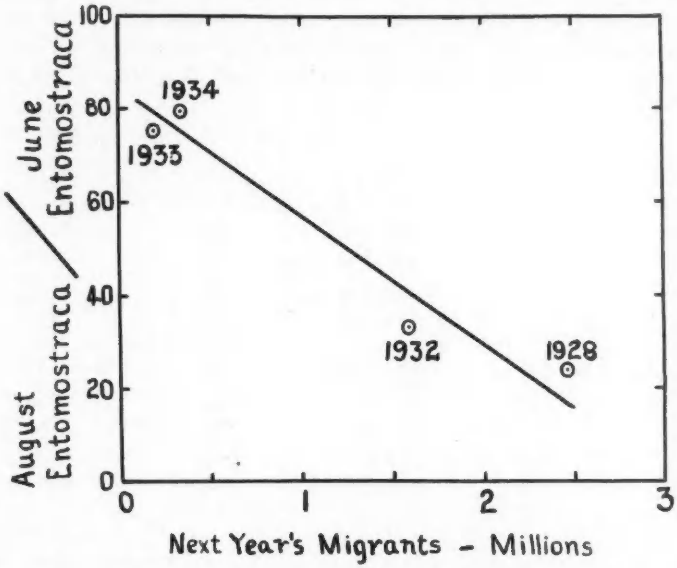

FIGURE 5. Relationship between the abundance of plankton Entomostraca in late summer, and the number of fingerling sockeye inhabiting the lake. The ordinate scale represents the mean abundance of Entomostraca in August, as a percentage of the mean abundance in June, for each year. The abcissa shows the number of fingerling sockeye migrants in the following year, which is here regarded as being approximately proportional to their abundance the previous summer.

1934. If the abundance of Entomostraca in May or June, before the sockeye are large enough to make serious inroads, be used to define the productive capacity of the lake for any given season, then the intensity of predation through the summer might be defined by the extent of their decrease by August or September. The effects of changing temperature and of the different species involved is minimized by considering June and August, calculating the entomostracan abundance in the later month as a percentage of that in the earlier, and comparing with the size of the sockeye population in different years. Postulating that the number in migration is proportional to their abundance the preceding summer, this relationship is illustrated in figure 5. It is evident that, in the four years concerned,

there is an inverse relationship between the size of the sockeye population and the abundance of Entomostraca in August. The correlation coefficient is calculated as -0.975, which is significant to a probability of about one chance in 50 (Fisher 1932, table 5A). It is possible that there is keen competition for food at seasons other than July-August-September; and, in fact, the rapid decrease in Entomostraca during autumn and winter might suggest it. However, in this case no relation can be demonstrated between the numbers of Entomostraca and numbers of sockeye, so that, although significant competition for the available food may exist, it is evidently not this predation which limits the abundance of Entomostraca at this season.

Much the best evidence of intraspecific competition for food is to be had from a comparison of the average size attained by yearling migrant sockeye with the number in migration. The following data are taken from Foerster (unpub. MS.).

		Yearling migrants	
Year	Total migrants	Aver. length (mm).	Aver. weight (grams).
1927	250,000	92	8.43
1928	338,000	81	5.04
1929	2,465,000	66	3.08
1930	105,000	88	6.54
1931	370,000	91	7.15
1932	789,000	90	7.34
1933	1,593,000	72	3.68
1934	185,000	86	6.50
1935	328,000	91	7.50
1936	503,000	95	8.84

From this it appears that in years of very large migrations ($1\frac{1}{2}$ to $2\frac{1}{2}$ million) the yearlings are relatively small, but that in years of moderate to small runs (800 to 100 thousand) there is no simple relationship between size of run and size of migrants. This is in accord with the view that competition for food must be severe enough to limit the growth of the sockeye of the largest populations.

In spite of the reduced growth, it has been shown (Foerster 1936b, p. 9) that even the smallest migrants do not suffer extra mortality in the lake. Nor is there evidence that they are handicapped in the struggle for existence during their later marine life. Hence it can be inferred that, in the years of small sockeye populations, the available plankton of the lake is either not being used completely, or not being used to best advantage. There seems no *nutritional* obstacle in the way of having the lake support, every year, fingerling sockeye populations considerably larger than the average, and possibly as large as the largest yet observed.

Another phase of intraspecific competition is that between the different year-classes. Evaluation of its significance is difficult, because of lack of exact knowledge of the relative numbers of fingerlings, yearlings and older sockeye living in the lake in any one year. The largest migrations of two-year-old smolts have followed the large yearling migrations, so it is in these years (1929-30, 1933-34)

that competition between the first and second year-classes would be likely to be most severe. The yearling migrants of 1930 and 1934 do not, however, appear to be so reduced in size as to be unusually small or otherwise handicapped.

Food Supply and Downstream Migration

It has sometimes been suggested that the seaward migration of young salmon is occasioned by a scarcity of food in their fresh-water home, so that they must start on a foraging expedition downstream in order to find nourishment in quantities sufficient to complete their growth. In this connexion table IV affords interesting information. It appears that sockeye migrating in April and early May do so at times when their food organisms are rapidly increasing in numbers, and are penetrating to greater depths than during the winter. A scarcity of food can hardly be the impelling force. It is true that the movement occurs just after the close of a long series of lean months, but it seems improbable that any cumulative malnutritional influence could move the sockeye to leave an environment where food organisms are increasing in size and abundance.

SUMMARY

The fingerling sockeye salmon of Cultus lake consume pelagic plankton almost exclusively, of the four genera *Epischura*, *Cyclops*, *Daphnia* and *Bosmina*.

The total amount of food found in stomachs, per unit bulk of the fish, varies throughout the year. It increases throughout the summer to late August, decreases greatly throughout the autumn and winter, and increases again in spring.

The kinds of plankton food eaten by fingerlings vary seasonally. When very small (May and early June) they contained chiefly *Cyclops* and *Bosmina*. Later in summer they take mostly *Daphnia*, with a few *Cyclops*. In autumn and winter *Cyclops* usually bulks largest, with at times significant numbers of *Bosmina*, or less often of *Daphnia*. *Epischura* is uncommon at all times.

Relative abundance in the lake is an important, but not the only, factor which determines the numerical proportions in which the four plankters occur in fingerling stomachs. The size of the plankter is important, those of larger size being more intensively exploited as a rule. Selection of a habitat outside the normal foraging range of the sockeye appears to diminish the extent of predation upon one species. The difference in relative consumption of *Cyclops* and *Bosmina* is apparently due to some other factor of availability or food preference, as none of the above seem adequate to explain it.

Sockeye in their second and third year of life also feed principally upon plankton, including relatively more of the larger species than the fingerlings take. In addition they consume significant quantities of midge pupae and terrestrial insects. A few of the largest ones contained very small fish.

Fingerling sockeye, held under conditions of fasting at 11.5-13.5°C., completed gastric digestion of a rather small plankton meal in four to eight hours.

The number of food organisms captured by sockeye held in the lake at a depth of 20 metres was very much less than by sockeye similarly held at 10 metres.

This and other considerations suggest that the principal foraging zone of fingerling sockeye in summer lies between 5 and 15 metres depth.

Extreme variations in the qualitative composition of the plankton food of sockeye held in a small space indicates that individual food preferences exist among them.

The total pelagic entomostracan food in the lake, as estimated from samples from a central station, varies seasonally, being greatest from May to July, least in midwinter. Variations in the average amount available in different years appear to occur, but the data do not demonstrate them with certainty. The annual variations in plankton populations are in any case very much less than the variations in sockeye populations.

At least eight other species of fish in the lake consume plankton at times. In summer, none of these occupy an open-water situation as do the sockeye, so interspecific competition for pelagic plankton is probably negligible at this time. The winter habitats and feeding habits of these fish are not well known.

Significant intraspecific competition for plankton food is suggested by the size of the standing crop in certain years, which is rather small in relation to the number of sockeye and their food consumption. It is suggested also by the fact that the available summer food decreased much more rapidly in years of large sockeye populations, than in others. It is confirmed by the significantly reduced growth rate of sockeye in those years.

Since the seaward migration of sockeye occurs at a season when the available food is rapidly increasing in amount, their movement out of the lake is probably not an effect of malnutrition.

ACKNOWLEDGEMENTS

As in a previous paper of this series, it is a pleasure to acknowledge the co-operation of Dr. R. E. Foerster, Chief Biologist of the Pacific Biological Station, throughout the course of the study, and to thank him for permission to cite data not yet published. The work also gains much from the timely criticisms made by Dr. W. A. Clemens, Director of the Station, and by other members of its staff.

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The Pigments of Salmon

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ABSTRACT

The red colour of the muscle flesh of the sockeye salmon (*Oncorhynchus nerka*) is due to the presence of two carotenoid pigments both of which have properties similar to those of astacin. Two red pigments were also found in the red muscle flesh of the steelhead salmon (*Salmo gairdneri*).

While the flesh of the majority of fishes is white, that of most species of salmon is orange-pink through orange-red to red. The nature of the red pigment in the Atlantic salmon, *Salmo salar*, has been the subject of several investigations. Krukenberg and Wagner (1885) found that the pigment could be extracted from red salmon muscle with hot alcohol, or alcoholic ether. They also observed that, when dissolved in carbon disulphide, it showed a single broad absorption band in the visible spectrum, extending approximately from the E to F Frauenhofer lines so that the maximum absorption would have been at approximately $500\mu\mu$. Newbigan (1898) also reported that the principal pigment in red salmon muscle was a lipochrome, or fat-soluble pigment. She concluded from her work that this red lipochrome readily formed sodium and potassium compounds, which could be decomposed with acetic acid. No other investigations were reported until that of Euler, Hellstrom and Malmberg (1933). They found that upon saponifying the ether extract of the red muscle flesh of the salmon, and extracting the saponified mass with ether, a flocculent precipitate separated at the alkali-ether interphase. When neutralized with acetic acid this material could be dissolved in benzene or pyridine. Its solution in the latter solvent showed a single absorption band in the visible spectrum with a maximum at $485\mu\mu$. They called the pigment "salmon acid" on account of its property of forming alkali metal compounds. It has since been shown by Emmerie, van Eekelen, Josephy and Wolff (1934) and Sorensen (1935) that this red pigment from salmon muscle is apparently identical with astacin, a carotenoid pigment which occurs in various marine organisms.

Of the several species of Pacific salmon (genus *Oncorhynchus*) which are caught in the coastal waters and streams of the Pacific coast, the sockeye (*O. nerka*) has in general the most deeply pigmented flesh. In addition to the *Oncorhynchi*, one species of the genus *Salmo*, the steelhead (*S. gairdneri*), is also taken in the commercial fishery of the Pacific coast. The work here reported embraces

a study of the pigments of the red muscle flesh of the sockeye and steelhead salmons.

EXPERIMENTAL METHOD

Extraction of Pigments

The red muscle flesh of the fresh fish was minced and thoroughly mixed. A portion of this was placed in a large flask and covered with alcohol which was then gently boiled for a few minutes. The alcoholic extract was decanted, an equal amount of fresh alcohol poured in, and the mixture again boiled. After thoroughly draining off the second alcoholic extract, the residue in the flask was finely pulverized in a mortar, put back in the flask, and extracted with successive portions of petroleum ether until the extracts were no longer coloured. The residue after extraction was very lightly coloured. All of the extracts were then combined and water added until all the pigment had been taken up by the petroleum ether. The alcohol-water layer was drained off and the petroleum ether solution was washed several times with distilled water, dehydrated with anhydrous sodium sulphate, and the solvent distilled off. The extract consisted of a dark red oil.

Characterization of Pigments

The extract of 100 grams of sockeye salmon flesh was saponified with alcoholic potassium hydroxide. After cooling, the saponified mass was diluted with water and shaken with ethyl ether in a separatory funnel. A red, flocculent precipitate similar to that described by Euler, Hellstrom and Malmberg (1933) separated out at the interphase. This was isolated from the rest of the material and neutralized with glacial acetic acid. It was soluble in excess of the acid, forming a blood-red solution from which the pigment was re-precipitated upon neutralization with solid potassium hydroxide. The free pigment was soluble in pyridine and slightly soluble in benzene.

The pigment which separated at the alkali-ether interphase was apparently only a small portion of the total pigment present, since the soaps were still coloured a deep red. For this reason, it was decided to resort to other means of separating the pigments.

Another portion of the extract of the red muscle flesh of the sockeye salmon was therefore subjected to chromatographic analysis by the method of Tswett (1906). The extract from 200 grams of the material was dissolved in petroleum ether and passed through a column of freshly-calcined alumina. The pigment was all retained in a single orange-red band at the top of the column. The oil, without a trace of colour, was recovered from the washings. When the column was washed with petroleum ether, the pigment moved very slowly down the column, separating into two bands. The adsorbent was pushed from the tube and the two pigments eluted separately with chloroform.

A similar portion of the extract of sockeye muscle was dissolved in petroleum ether and passed through a column of calcium carbonate. The pigment was adsorbed by the calcium carbonate, upon which it separated into two bands on continued washing with the solvent as it did on alumina. The calcium carbonate

did not, however, hold the pigment as strongly as the alumina, both bands moving down the column much more rapidly when the former was employed. The washing was continued until both the pigments had been washed from the column, after which they were collected separately for spectroscopic examination. The more firmly-retained pigment showed a single maximum in the visible spectrum at $490\mu\mu$, and the less firmly-retained pigment a single maximum at $500\mu\mu$, each in carbon disulphide solution.

Another sample of the sockeye muscle extract was saponified with colourless 20 per cent alcoholic potassium hydroxide. The saponified material was diluted with distilled water and shaken with petroleum ether, but the solvent did not take up any pigment. Glacial acetic acid was then added until all of the pigment migrated into the petroleum ether phase. This was drawn off separately, washed several times with distilled water, dehydrated with anhydrous sodium sulphate, and then concentrated to a small volume by distilling off the petroleum ether. This concentrated solution of the pigment and fatty acids in petroleum ether was passed through a column of alumina. The pigment was retained in a single band at the top of the column. Washing with petroleum ether caused this band to spread out somewhat, but it did not separate into discrete bands. When, however, glacial acetic acid was passed through the column it rapidly washed out one pigment, leaving another adsorbed at the top of the column. Further washing with acetic acid did not cause this latter band to move appreciably, but it was successfully eluted with chloroform. The visible absorption spectrum of both the free pigments, dissolved in carbon disulphide, had a single maximum at $500\mu\mu$.

A brief study was also made of the pigments of the red muscle flesh of the steelhead salmon (*Salmo gairdneri*). A sample of the pigmented extract, prepared as described above and dissolved in petroleum ether, was passed through a column of alumina. The pigment was all held by the adsorbent at the top of the column, and gradually separated into two bands when the column was washed with petroleum ether. The lower band was washed from the column with petroleum ether. The upper was eluted with chloroform.

DISCUSSION

The properties of astacin have been summarized by Zechmeister (1934). It occurs in the form of esters which are frequently found in combination with protein. The astacin esters are split off from the protein by the action of alcohol, and can themselves be decomposed by strong alkalies. Astacin itself, however, then combines with alkali to form a compound from which the free pigment can be liberated with acetic acid. It has a single absorption band in the visible spectrum. Lederer (1935) gives the absorption maximum in carbon disulphide solution as $510\mu\mu$; Sorensen (1935) as $506\mu\mu$.

It is evident from the properties studied that both the sockeye pigments are closely related to astacin. The possibility of these two pigments being different esters of astacin has been ruled out, since two separate pigments were still found after saponification and neutralization with acetic acid. It is possible that they may be isomeric forms of astacin.

An extended study was not made of the pigments of the red muscle flesh of the steelhead salmon, since it was desired only to find out if two pigments were present in the flesh of a fish of the genus *Salmo*, as in that of a fish of the genus *Oncorhynchus*. Since the steelhead pigments were similar in their adsorption-behaviour to those of the sockeye, it is probable that they are identical with them,

It is worthy of note that astacin apparently cannot function as a precursor of vitamin A in mammalian nutrition. While 3 to 4γ of carotene daily, administered to rats fed an otherwise vitamin A-free diet, is sufficient to bring about normal growth, Kuhn and Lederer (1933) found that 30γ of astacin per day produced no growth-effect at all. It has also been reported from these laboratories by Bailey (1936) that two highly pigmented samples of salmon flesh oil contained respectively only 2.0 and 2.5 international units of vitamin A per gram.

SUMMARY AND CONCLUSIONS

The red muscle flesh of the sockeye salmon (*Oncorhynchus nerka*) contains two red pigments, both of which have properties similar to those of astacin.

The red muscle flesh of the steelhead salmon (*Salmo gairdneri*) contains two red pigments. From their adsorption behaviour they appear to be identical with those of the sockeye salmon.

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Some Characteristics of the Enzymes of the Pyloric Caeca of Cod and Haddock

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ABSTRACT

Investigation of fish enzymes for leather bates reveals that those of the pyloric caeca show their greatest influence on casein and collagen at hydrogen ion concentrations of approximately pH 8. The protease showed its maximum activity towards casein at a temperature of 45°C. Ammonium salts at certain concentrations increased the rate of hydrolysis of collagen by about 40 per cent, but had no like stimulating effect on the hydrolysis of casein. A comparison showed that pyloric caeca enzymes were just as satisfactory as commercial leather bates or hog pancreas. When the pyloric caeca are allowed to autolyse at room temperature, the protease activity is constant for the first 24 hours, declines rapidly during the next 80 hours, and slowly thereafter. The most suitable method for preparing a dried preparation was by evaporation under partial vacuum, which, however, is accompanied by some loss of activity.

This paper describes certain work which has been carried out on the enzymes of the pyloric caeca of cod and haddock with the object of furnishing other laboratories with data to enable them to assess the value of such material in leather bating. This examination was undertaken on the suggestion of Dr. G. S. Whitby, Director, Chemistry Division, National Research Council, Ottawa.

Among others a bate must possess the property of digesting certain skin constituents undesirable in the finished leather. Wilson (1928, p. 340) states that bating to remove all the elastin fibres is desirable in the production of an extremely soft leather, but adds that the really important function in bating seems to be the digestion of keratose. Wood (1921) is of the opinion that it is not desirable to remove all the elastin fibres, but he agrees that they must be weakened to a certain extent to impart the desired suppleness to the finished leather. Wilson and Merrill (1926) feel that one of the most important functions of the enzymes in bating is the hydrolysis of keratose. Accordingly a desirable bate in so far as its enzyme content is concerned is one which selectively attacks keratose and elastin, but is inert towards collagen, the chief leather forming element in hides. While such an ideal preparation is not available, a bate which has much more to recommend it than the old dung bates formerly employed was prepared by Röhm (1908). His preparation consisted of an aqueous extract of pancreas diluted with an 0.1 per cent solution of ammonium chloride. His patent also called for the preliminary precipitation of any carbonic acid in the bating liquors with lime water.

A number of blind tube-like sacs attached near the pyloric end of the stomach,

and apparently serving in a capacity similar to the pancreas of the mammal, are found in certain fish and are known as the pyloric caeca. It was to determine the suitability of these glands for the preparation of a leather bate that this investigation was undertaken.

In the eastern Maritime provinces of Canada 1,530,000 cwt. (70,000,000 kg) of cod and haddock were landed in 1934. Experiment has shown that the pyloric caeca represent on an average about 1.1 per cent of the weight of the fish as landed.. If dried in a vacuum oven, 100 lb. (45 kg.) of the pyloric appendages would yield about 22 lb. (11 kg.) of a dry bate. Hence the possible annual production of bating material from this source in the eastern Maritime provinces would amount to about 3,300 cwt. (150,000 kg.). The potency of the material is from 5 to 8 times that of the ordinary commercial bates.

METHODS

Preparation of "Standard" Enzyme

Dried preparations of the pyloric caeca of cod or of cod and haddock have been made in a number of ways. A crude enzyme preparation made according to the method described immediately below will be referred to in the following pages as the "standard" preparation, and all experiments, except where otherwise stated, were carried out with this type of dried enzyme. Other methods of preparing the enzyme are dealt with at appropriate places in subsequent pages. The "standard" preparation was made in the following manner. The pyloric caeca were cut from fresh fish, left in a freezing room over-night and ground the next morning before allowing them to thaw. The ground tissue was then treated with 1.5 times its weight of acetone to which was added about 10 per cent of ether, and the whole allowed to stand about half an hour. The residue was squeezed as dry as possible and again treated with acetone and ether in the same proportions. After standing about half an hour the residue was again squeezed, then spread out and allowed to dry at room temperature. After drying, the caeca were ground to pass a 20 mesh screen yielding a yellowish powder with a solubility in water of about 30 per cent. A "solution" of the preparation is slightly yellowish and somewhat opalescent. In addition to its tryptic and lipolytic activity, which will be considered in the following pages, the solution possesses some peptic and rennet activity. No amylase activity could be demonstrated.

Measurement of Activity

The value of an enzyme as a bating material lies more in its ability to dissolve certain protein constituents of the hide than in any subsequent hydrolysing effect it might have on the dissolved proteins. Accordingly an attempt has been made in the present work to assess the protease activity of the enzyme by measuring the early observable stages in protein decomposition rather than increases in amino nitrogen, which are a measure of later hydrolytic rupture. Farber and Wynne (1935) have shown that in the hydrolysis of casein 50 per cent was rendered

soluble in trichloracetic acid although in the same time amino nitrogen had accumulated to only slightly more than 1 per cent of that present after two weeks' action. Moreover, during the first part of the reaction the classical laws governing enzyme kinetics are applicable with fair accuracy, but during the later phases they are masked by such disturbing factors as combination of the enzyme with reaction products, reverse reactions, and destruction of the enzyme itself. Therefore it becomes impossible to gauge the true value of an enzyme for bating purposes by any method which does not eliminate these factors.

As has been pointed out by many authors, conclusions drawn from the activity of an enzyme toward casein may be very misleading in evaluating its activity on the skin constituents which it must attack. In spite of this, because casein can be obtained in a relatively pure or at least in standard condition, it has remained customary to determine the value of a bate by its action on this substrate, and accordingly this protein has been used as a standard throughout this series of experiments. Enzyme activity on casein or keratose was determined in the following manner. Two and a half grams were dissolved in a buffer solution and the volume of substrate made to 50 ml. The enzyme dissolved in 10 ml. of water was added and a 5 ml. sample of the digest immediately withdrawn and added to 10 ml. of 10 per cent trichloracetic acid. After various periods of from 15 to 120 minutes additional samples were withdrawn and treated likewise. The precipitated protein was filtered from the trichloracetic acid and nitrogen determinations made on 5 ml. of the filtrate. The nitrogen value of the sample at zero time was subtracted from the subsequent values, and from a smooth progress curve the increase in nitrogen soluble in trichloracetic acid after any given period determined. In all cases, except where otherwise mentioned, the digestion took place at a temperature of 40°C. and a reaction of pH 8.0.

When dealing with collagen or elastin the powder was suspended in the buffer solution used, and as soon as the enzyme was added samples were removed and filtered. After various intervals samples were again removed, filtered and nitrogen determinations made on portions of the filtrate. A series of such determinations was made just as in the case of casein, a smooth curve was drawn and the activity of the enzyme measured by the increase in the amount of soluble nitrogen. Measurement of lipolytic activity was carried out by adding to a series of flasks containing 3 ml. of olive oil 10 ml. of the enzyme solutions prepared by extracting the dried powder with a tenth molar ammonia—ammonium chloride buffer of pH 8.0. After each of several increasing time intervals at 40°C. one flask of the series was titrated with tenth normal alkali in the presence of 50 ml. of a solution made up of equal parts of benzene and alcohol. Twelve drops of phenolphthalein were used as an indicator. After subtracting blanks from such determinations, a smooth progress curve was drawn from which the increase in free fatty acid after any given period could be determined. In all cases, enzyme activity was expressed from values taken from the progress curve during the early stages of the reaction before such factors as inactivation of the enzyme and depletion of the substrate began to make themselves felt.

SUBSTRATES

The casein used throughout these experiments was Merck's, prepared according to Hammarsten. The collagen substrate was prepared from American standard hide powder. The keratose used was obtained from Baird and Tatlock and was described as keratin prepared with pepsin. It was completely soluble in phosphate buffers of pH 8 and was precipitated by an acetate buffer of a reaction of pH 4. Elastin was prepared from the *ligamentum nuchae* by treatment with salt, potassium hydroxide and acids after the manner described by Wilson (1928).

EXPERIMENTAL

Influence of Acidity on Activity

Practically all enzymes are influenced by the reaction of the medium in which they act. In this regard the proteolytic enzymes of the pyloric caeca are no exception. In figure 1 is shown the number of milligrams of casein nitrogen made soluble in trichloracetic acid after one hour's digestion by 1 g. of the "standard" preparation at the pH values indicated. The various hydrogen ion concentrations of the digests above pH 7 were maintained with suitable buffer solutions of sodium carbonate and sodium borate; and below pH 7 with suitable disodium phosphate and citric acid buffer solutions. The digestion was carried out at a temperature of 40°C.

In addition to the activity of the enzyme preparation toward casein, figure 1

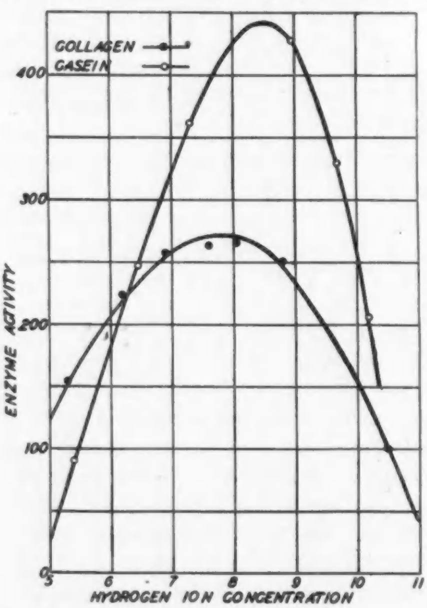

FIGURE 1. The relation between hydrogen ion concentration and activity of the pyloric caeca protease of the cod in Mg.

also shows the activity of the product toward collagen. When collagen was used as a substrate the reaction of the digest was maintained with mixtures of primary, secondary and tertiary phosphates at a concentration of tenth molar. Figure 1 shows the amount of collagen nitrogen rendered soluble in water by 1 g. of the powder after a reaction period of 20 minutes. Since collagen is slightly hydrolysed by water at a temperature of 40°C., it was necessary to run blanks along with the experimental flasks. The blank values were subtracted from the experimental values and the corrected figures are plotted in figure 1.

It will be seen that the optimum hydrogen ion concentration for casein hydrolysis is about pH 8.5, that for collagen about pH 8.0. Examination shows that the range of optimum activity for collagen is quite broad, being much wider than that for casein. Strather and Machon (1931), with an enzyme from fish guts prepared in a different manner from the one used in these experiments, found an optimum pH value for the hydrolysis of collagen of about 8.0, but their enzyme exerted its greatest activity over a much narrower range.

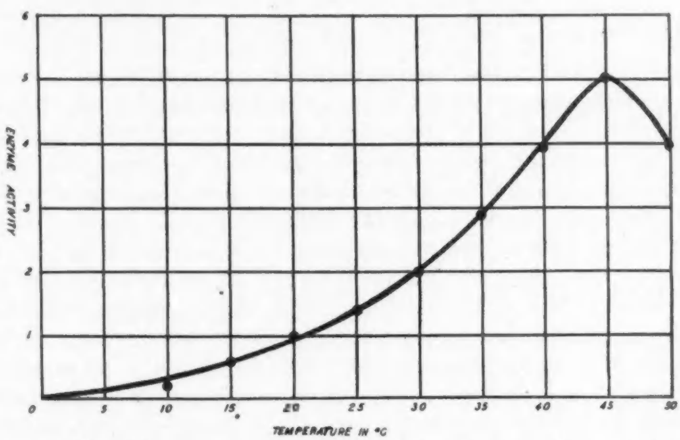

FIGURE 2. The effect of temperature on the activity of the pyloric caeca protease of cod.

RELATIONSHIP BETWEEN TEMPERATURE AND ACTIVITY

The velocity of enzyme reactions is increased up to a certain point as the temperature increases. On further increase the reaction velocity diminishes due to the destructive action of high temperatures on the enzyme. Because of the fact that *in vivo* the enzymes of fish must function at low temperatures, it has been suggested that they might show a low optimal temperature. To determine the validity of this hypothesis, activity experiments were carried out at 5 degree intervals over the range from 10 to 50°C. In figure 2 is shown the relative increase in nitrogen soluble in trichloracetic acid after one hour's hydrolysis at various temperatures. Casein in a borate carbonate buffer at pH 8.5 was used as a substrate in this experiment.

Examination of figure 2 shows the maximum activity at a temperature of

45°C., the velocity of hydrolysis being about 25 times greater at this temperature than at 10°C. Above 45°C. the enzyme begins to lose its activity quite rapidly. Most enzymes are not rapidly inactivated at as low a temperature as this, but since an optimum temperature can be affected by so many factors, it is difficult to draw any conclusions from the observation. In this respect, however, the enzyme preparation used in this series of experiments differed from that of Strather and Machon (1931) in that they found no inactivation below 55°C.

From the values shown in figure 2 the Q_{10} values for the tryptic enzyme from the plyoric appendages have been calculated, as follows:

Temp. range (°C.)	10-20	15-25	20-30	25-35	30-40	35-45
Temp. coeff.	3.0	2.5	2.2	2.0	1.9	1.8

The Q_{10} values, as is usual with hydrolytic enzymes, show a falling off as the temperature range increases. The coefficients are also lower than the coefficients often quoted for trypsin, but are a little higher than those calculated from Strather and Machon's work on collagen.

Effect of Ammonium Salts on Activity

Röhm's original commercial bate consisted of an aqueous extract of pancreas in a dilute ammonium chloride solution. It has been the practice ever since to include various proportions of ammonium salts in the different commercial preparations. One commercial sample examined was found to contain 58.2 per cent of ammonium chloride and 33 per cent of an insoluble filler. It is apparent, therefore, that an investigation of this nature should include a study of the effects of ammonium salts on the hydrolysis brought about by the enzymes of the pyloric caeca. Employing a weak pancreatic extract Wilson (1928, p. 337) has shown that a concentration of M x 10^{-2} ammonium chloride increases the rate of removal of elastin from calf skin. At higher concentrations the rate of elastin removal decreases until at a concentration of 10 M x 10^{-2} the activity of his enzyme falls to zero. Kubelka and Wagner (1929) using their method, which consists in determining the enzyme concentration necessary to hydrolyse casein under certain specified conditions sufficiently so that it will no longer precipitate at its isoelectric point, have shown that the activity of a pancreatic preparation was almost tripled when the enzyme was mixed with an equal weight of ammonium sulphate. Stiasny (1929) cautions that neutral salts may exert an influence on the substrate of at least as great significance as that exerted on the enzyme itself. He points out that there is a distinct relationship between the swelling ability of the salt and the promotion of enzyme activity. Nauen (1931) has found an almost linear relationship between the swelling of collagen produced by various salts and its rate of proteolysis. Similarly Kuntzel and Dietsche (1931) have found that the presence of ammonium sulphate increases the activity of pancreatin toward collagen, but state that the activation by neutral salts is essentially a matter of the swelling of the collagen. In the course of this investigation the effect of ammonium salts on the digestion of casein and collagen has been studied with somewhat confusing results. As already pointed out, Kubelka and Wagner have shown ammonium sulphate to

have a stimulating effect on the enzymic digestion of casein. Parallel experiments using their method and applying it to the "standard" preparation of the pyloric caeca showed similar results. One enzyme preparation in the absence of any ammonium salt showed a casein number of 660. When mixed with an equal weight of ammonium sulphate the casein number rose to 2,100. The results of one experiment are shown in table I.

TABLE I. Influence of ammonium sulphate on the digestion of casein

Per cent of salt to enzyme	Concentration of salt in digest $M \times 10^{-3}$	*Casein number of enzyme
0.0	0.0	660
33	0.50	1250
50	0.57	1670
100	0.91	2100

*The casein number is the number of grams of casein digested by 1,000 g. of bate in 30 minutes at 40°C. The term "digested" as applied to the Kubelka-Wagner procedure implies that the casein is no longer precipitated at its isoelectric point by an alcoholic acetic acid solution.

As is shown above, and as Kubelka and Wagner found with their pancreatic extracts, ammonium salts appear to increase the rate of breakdown of casein. Experiments carried out with an extract of the "standard" preparation on casein in the presence of both ammonium chloride and ammonium sulphate, and employing the trichloracetic acid technique, failed to confirm the findings shown in table I. Ammonium sulphate in the digest at concentrations of 15.6, 31.2, 62.5, and 125 $M \times 10^{-3}$ showed no stimulating effect on the rate of breakdown of casein when using this technique. Similarly ammonium chloride when present in a casein digest at concentrations of 5, 25, 80, 250 and 1,000 $M \times 10^{-4}$ neither increased nor retarded the rate of casein decomposition as measured by this method. Digestion was carried on at a hydrogen-ion concentration of pH 8.95 and a temperature of 40°C.

In addition to studying the influence of ammonium salts on the enzymic hydrolysis of casein, the effect on the hydrolysis of collagen has been examined. All experiments were carried out at 40°C. and pH 8.3. Results of this series of experiments are shown in figure 3.

Figure 3 shows the increased percentage of hydrolysis of collagen by the enzymes of the pyloric caeca as a function of the molar concentration of ammonium chloride present in the digestion mixture. The stimulation was calculated from the amount of hydrolysis found in the control and experimental flasks after a digestion period of 30 minutes. Under the experimental conditions employed about 30 per cent of the collagen present in the control was digested during this period.

From this study of the effect of ammonium chloride on hydrolysis two facts become apparent. The presence of ammonium salts does not increase the rate of hydrolysis of casein to fractions lower than those precipitated by trichloracetic acid; and, although ammonium chloride at certain concentrations does increase the rate

of collagen breakdown by the enzymes from the pyloric caeca, it exerts far less effect than one would be led to suppose from the activity of the bate on casein according to either the Kubelka and Wagner or Fuld and Gross methods (see Wilson 1928, p. 379). It is also apparent, as was shown by Wilson, that high concentrations of ammonium salts tend to inhibit the action of the enzyme itself.

Comparison with a Commercial Bate and Dried Hog Pancreas

As previously pointed out, the consensus of opinion as to the function of an ideal bate is the removal of keratose residues and the softening or removal of elastin fibres. In addition, although little critical work has been done on the action of pancreatic enzymes on the fatty constituents of the skin, certain investigators feel that some lipolytic activity is desirable particularly in the treatment of sheep skins. An experiment was therefore carried out to determine if the enzymes of the pyloric caeca would meet these requirements as satisfactorily as the bates now commonly employed. Accordingly the quantity of "standard" preparation of hog pancreas and of commercial bate necessary to increase the non-precipitable casein nitrogen in an aliquot of the digest by the arbitrarily fixed amount of 1 mg. in 20 minutes was determined. (Under the experimental conditions employed this represents a casein digestion of about 10 per cent). These quantities of the different enzyme preparations were then allowed to act on collagen, elastin, kera-

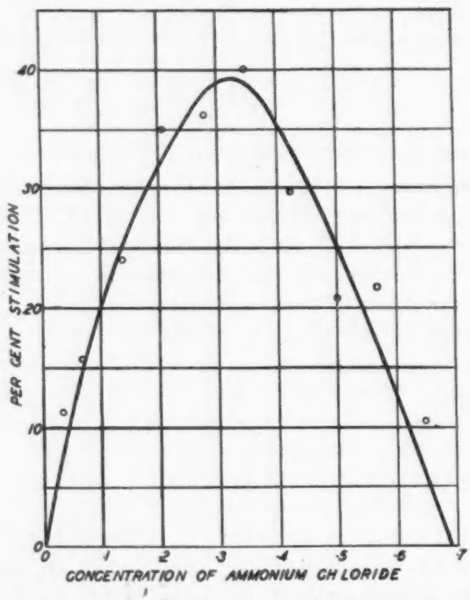

FIGURE 3. The effect of ammonium chloride on the hydrolysis of collagen by the pyloric caeca protease.

tose, and olive oil under similar experimental conditions and the amount of the substrate digested was determined. These values are shown in table II.

Table II shows that all the preparations employed attacked collagen much more rapidly than either casein, elastin or keratose. It shows also that the enzyme of the pyloric caeca acted similarly to those of the hog pancreas in their attack on the various substrates. The commercial bate used contained about 60 per cent of ammonium chloride; as shown by the results appearing in figure 3, the presence of this salt accelerates the rate of breakdown of collagen, thus accounting for the fact that the commercial bate was more active toward elastin and collagen than the other preparations. The conclusion therefore seems justified that in so far as the relative activity of the enzymes toward the various substrates which they must attack as leather bates is concerned, those of the pyloric caeca of fish should prove just as satisfactory as those of the hog or those now employed by the leather industry.

Effect of Autolysis on Enzyme Preparation

In the Maritime provinces of Canada several days may elapse between the time fish are caught and the time they reach shore. The pyloric caeca of such fish would therefore autolyse considerably before reaching land where they could be

Table II. Relative rates of hydrolysis of various substrates by different enzyme preparations

Enzyme	Mg. increase in soluble nitrogen				Mg. increase in oleic acid olive oil
	Casein	Elastin	Collagen	Keratose	
Commercial bate	1	0.10	3.9	0.11	...
Hog pancreas	1	0.06	3.3	0.15	.66
Pyloric caeca	1	0.05	3.1	0.14	.86

converted to a dried preparation. Therefore it was thought advisable to carry out preliminary experiments on the relationship between the length of the period of autolysis and the activity of the autolysed caeca, and to give consideration to the relationship between the period of autolysis of the caeca and the yields and activity of a "standard" preparation subsequently prepared from them. In the first experiment 100 g. of frozen ground caeca were treated with 500 ml. of water and after thorough maceration the mass was centrifuged and the activity of the supernatant liquid on casein was determined. The same procedure was repeated after increasing periods of autolysis under toluene at room temperature (about 22°C.). After an autolysis period of 48 hours it was unnecessary to centrifuge the mass, as all the large particles had become dissolved and the mass had become homogeneous. Figure 4 shows the amount of casein made soluble in trichloracetic acid in 20 minutes at 40°C. by 10 ml. of the liquid after increasing periods of autolysis.

It is seen that autolysis for a period up to 24 hours results in little falling off

in the activity of the enzyme. This is followed by a period of rapidly decreasing activity until the preparation retains only about one-third of its original potency, after which its activity declines slowly.

In the second series of experiments to establish the relationship between the periods of autolysis and the yields and activity of the "standard" preparation made ments the pyloric caeca as received at the laboratory (they were about 12 hours from them, only the earlier stages of autolysis were considered. In these experi-old, during which time they had been stored on ice) were allowed to autolyse for

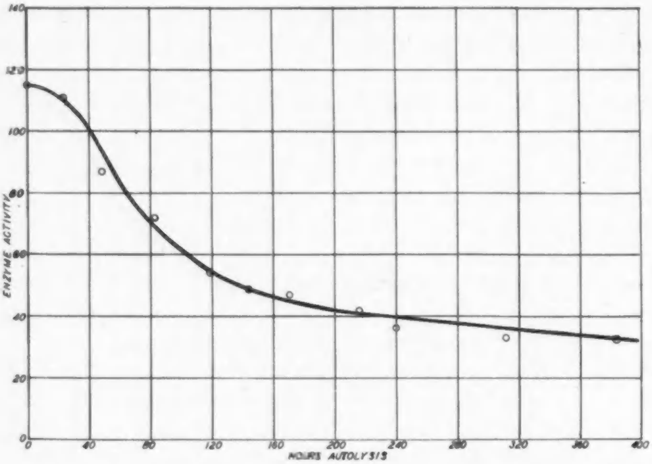

FIGURE 4. The effect of autolysis on the activity of the pyloric caeca protease.

increasing periods at room temperature. They were then treated with acetone in the same concentration as used in the preparation of the "standard" enzyme and dried. The yields and the activity of the different preparations were then determined. The values from two sets of experiments are shown in table III.

The second column of the table shows the yield of dried enzyme from 100 g.

TABLE III. Effect of autolysis on yields and activity of pyloric caeca

Hours autolysis	Enzyme yield (g.)	Increase in soluble N by 1 g. in 20 minutes (mg.)	Yield in units
0	15.5	104	1620
5	14.8	132	1940
15	14.0	134	1870
23	11.2	126	1410
0	16.5	151	2500
7	15.0	256	3840
24	9.5	209	1980

of wet caeca after treatment with acetone. It is to be noted that as the period of autolysis lengthens, the amount of dry enzyme prepared by the "standard" acetone treatment decreases. The third column expresses the calculated number of milligrams of casein nitrogen made soluble in trichloracetic acid in a period of 20 minutes by 1 g. of the different enzyme preparations. It is interesting to note that 1 g. of the commercial bate examined increased the casein nitrogen under parallel experimental conditions by 24 mg. It is therefore apparent that the most active of the above preparations could be diluted 10 times with a filler and still produce a bate comparable in activity to the commercial bate examined. It will be observed that slight autolysis increased the activity per gram in both sets of experiments. Even after 24 hours autolysis the activity per gram of the dried preparation was greater than that of the product made from fresh caeca. The figures in the last column in table III were obtained by multiplying those in columns 2 and 3 and show what we have chosen to call unit yields. They therefore represent, in each case, the activity of what was originally 100 g. of wet caeca after various periods of autolysis followed by the "standard" acetone treatment. It is apparent from this column that a period of autolysis at room temperature of around 10 hours increases the number of units of activity which can be prepared from the caeca by the "standard" method. Although as may be seen from figure 4 periods of autolysis up to about 40 hours do not greatly diminish the activity per gram of the dried preparation, yet the yields obtained by the "standard" procedure diminish and in consequence the yield in units suffers considerably.

Methods of Drying Pyloric Caeca

In order to learn the most suitable concentration of acetone to obtain the highest yield of the greatest activity and thus the highest unit yield a series of preparations was made using varying proportions of acetone. Results of this investigation in the case of two lots of caeca are shown in table IV.

The products were made by employing the same technique as for the "standard"

Table IV. Effect of various acetone concentrations in preparing the enzymes

Ml. of acetone added to 100 g. of caeca	Enzyme yield	Increase in soluble N by 1 g. in 20 minutes (mg.)	Yield in units*
50	14.7	32.	467
100	15.5	105	1620
150	15.5	167	2590
50	16.1	59	950
150	16.2	101	1640
250	15.8	151	2390
350	16.8	174	2920
450	21.2	122	2590

*This is the product of enzyme yield and increase in soluble nitrogen.

preparation, the only variable being the quantity of acetone added to the wet caeca. Although the activity per gram between the two lots differs considerably, the table shows that while the yield in grams remains fairly constant over most of the range of acetone concentrations employed, yet the activity per gram of the dried preparation increases steadily up to the point where the acetone concentration reaches about 80 per cent (100 g. of caeca contain approximately 80 g. of water). At higher concentrations the yield of dry material increases but the activity per gram decreases more than sufficient to offset this, with the net result that the yield in units begins to decrease.

Two other types of crude dried enzymes were prepared in the course of this investigation: (1) an aqueous extract of the caeca was dried under vacuum at a temperature of 40°C.; (2) the caeca were simply ground and dried in a vacuum oven at 40°C.

One hundred g. of the ground caeca were extracted twice with 100 ml. portions of distilled water. The extract was centrifuged and the centrifugate concentrated and finally dried at a temperature of 40°C. under reduced pressure. A yield of 10 g. of dried extract was obtained from 100 g. of caeca. The residue after centrifuging was treated with acetone and dried at room temperature. Its yield amounted to 10.8 g. One g. of the dried extract increased the amount of non-precipitable nitrogen from casein by 238 mg. in 20 minutes at 40°C. One g. of the dried residue under the same conditions increased casein soluble nitrogen by 61 mg. One hundred g. of the same lot of caeca after treatment with acetone according to the "standard" procedure yielded 16.5 g. of a dry preparation, 1 g. of which increased the casein soluble nitrogen by 151 mg. under comparable conditions.

One hundred g. of the pyloric appendages were ground and placed in a vacuum oven the walls of which were maintained at 40°C. The caeca became dry in about 10 hours. The yield obtained in this manner was 23.6 g., one of which increased the non-precipitable casein nitrogen by 166 mg. in 20 minutes. The yield in units was therefore 3,910. This enzyme preparation was made from the same lot as those dealt with in the second part of table IV. From this table it is seen that the highest yield in units obtained by any concentration of acetone was only 2,920 or almost 30 per cent lower than one obtains by simple vacuum drying. The vacuum dried preparation of ground caeca was approximately 7 times as potent as the commercial sample examined.

SUMMARY

A study of the proteolytic enzymes of the pyloric caeca of codfish has shown that they exert their greatest effect at about the same hydrogen ion concentration as trypsin.

In addition to its tryptic activity a dried preparation of the pyloric caeca showed the presence of a lipase and a low peptic and rennet activity. No amylase was found.

The relationship between the activity of the pyloric caeca enzymes and tem-

perature was determined. At temperatures above 45°C. the enzyme becomes inactivated.

It was found that ammonium salts increased the rate of hydrolysis of collagen by the pyloric caeca enzymes by about 40 per cent at certain concentrations but had no like effect on the hydrolysis of casein.

A comparison between a commercial bate, hog pancreas, and the enzymes of the pyloric caeca in their action on casein, collagen, elastin and olive oil showed that all behaved similarly. This leads to the conclusion that, in so far as their proteolytic and lipolytic enzymes are concerned, the pyloric caeca would furnish a suitable material from which to prepare a leather bate.

Some consideration has been given to the effect of autolysis on the preparation of a dried product from the pyloric caeca. It was found that a moderate amount of autolysis was not particularly injurious.

A preliminary study was made of different methods of preparing a dried product.

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